Effects of excess root applied silicon on *Triticum aestivum* and *Zea mays* under *Aphis gossypii* and *Schistocerca gregaria* herbivory and water-stressed conditions.

---

by

Sudakshina Roy Deluca

Presented for the degree of Master of Philosophy

School of life science, University of Sussex

April 2012

I hereby declare that this thesis has not been submitted, either in the same or different form to this or any other University for a degree.

Signature:

g:\pennyk\unisussexdeclaration.doc

Acknowledgments

Firstly I would like to thank my supervisors Libby John and Sue Hartley; their help and guidance during this M.Phil has been invaluable.

I would like to thank all my friends and colleagues at the University of Sussex for their encouragement and advice over the last few years. I would especially like to thank Claudia, both for her friendship and for all her help.

I would also like to thank my sister and the rest of my family for their encouragement, interest and patience in my endeavours. I am very grateful to my parents (Mr. Sachindra Nath Roy and Mrs. Arati Roy), my dear untie (Miss Kanika Roy), and 'Sai Baba' for their financial help and their blessings. My one year old son Ethan, born while I have been working towards this M.Phil, provides me with all the inspiration I should ever need. Finally and most importantly, I would like to thank my husband Carlo for his support, especially during the difficult times; without him I would not have come so far.

Table of Contents

Abstract

Triticum aestivum (wheat) and *Zea mays* (maize) are two of the most important staple food and industrial crops used by developed and developing countries. Drought and pest attack often reduces wheat and maize production, causing huge economic losses. Silicon has been proposed to protect plants from several biotic and abiotic environmental stresses such as pest attack and drought. Silicon accumulation in plants can increase the abrasiveness of their leaves, potentially deterring herbivory by several important pest species, such as *Aphis gossypii* (aphids) and *Schistocerca gregaria* **(**locusts).

Silicon accumulation in plants may also reduce transpiration rates and thus increase their drought tolerance. Here, the potentially protective effects of root silicon application to *Triticum aestivum* and *Zea mays,* against both water stress and herbivory by *Aphis gossypii* and *Schistocerca gregaria*, were investigated in a series of greenhouse experiments. $Na_2SiO_3.9H_2O$ (Sodium Meta Silicate) was used as a source of silicon. Experiments manipulated the impact of silicon on drought and controlled plants with and without pest species present; the influence of silicon application on herbivore performance and its potential interaction with water stress was also investigated. Aphid performance was evaluated by determining the change in the population growth rate over two weeks. The influence of silicon application on locust herbivory was determined by calculating the total damage to the plants over two weeks. Silicon application increased the abrasiveness of the leaves of both *Triticum aestivum* and *Zea mays*, and enhanced their resistance to *Schistocerca gregaria* herbivory; however the increased abrasiveness did not have an effect on *Aphis gossypii*

performance. Additionally silicon accumulation enhanced drought tolerance when the temperature was kept between 20-25 °C, but it had no effect on plant biomass and plant photosynthetic rate when the green-house temperature was maintained between 40-45 °C. Silicon application to crop species provides a potentially cost-effective alternative to pesticides, and may increase drought tolerance in both C3 and C4 crop species.

Chapter 1: General introduction and literature review

1. Introduction

1.1. Introduction to *Triticum aestivum* and *Zea mays* plants

T. aestivum and *Z. mays* are the two main crops used by both developed and developing countries. In 2011, the world's total wheat production was approximately 676 million tonnes from the start of the year until the record was taken on 23rd March, which exceeds the total for the same period in 2010 by 3.4% (http://www.fao.org/news/story/en/item/53813/icode/23rd March, 2011). *T. aestivum* is planted on more than 240 million ha and almost 1billion people from developing countries depend on it. Maize is cultivated in the tropics, sub tropics and temperate regions. Nowadays almost 70 countries, including 15 developed and 58 developing countries use maize as a staple diet. In 2011/ 2012 world's maize production was 847 million tonnes (http://www.topcommodities.net/2011/04/igc-forecast-world-maize production.html/ 11th April, 2012). 22 of 145 countries have a maize consumption of more than 100g/person/day (www.fao.org, 2011).

T. aestivum and *Z. mays* both belong to the taxonomic family Poaceae, but they differ in their photosynthetic pathways. *T. aestivum* is a C3 plant. C3 is the most common photosynthesis pathway where plant uses single chloroplasts to convert light energy to chemical energy (Robert *et al.*, 1995). In general C3 plants contain higher protein content than C4 plants; C3 plants contain 10-20 % protein by dry weight whereas C4 plants contain approximately 5-10 % (Robert *et al.*, 1995).

Z. mays is a C4 plant that initially converts CO_2 to four carbon molecule carbon dicarboxylic acid oxaloacetate, during photosynthesis. C4 plants are generally drought tolerant plants as they can reduce photorespiration by accumulation of CO_2 (Robert *et al.*, 1995).

The extent of plant damage by herbivores may also be dependent upon the photosynthetic pathways of the plant. Herbivores are thought to prefer C3 more than C4 plant species (Boutton *et al.*, 1978). Caswell and Reed (1975) demonstrated that some grasshopper species are not able to digest C4 plants because of their anatomical structure. The bundle sheath cells of C4 plants consist of very thick cell walls, which protect the protein and starch-rich stores. This makes C4 plants potentially more resistant to physical disruption by herbivory, by lowering herbivore fecundity and survival rate (Caswell et al., 1973; Boutton *et al.*, 1978).

1.2 Drought

Drought, defined as a meteorological phenomenon, is a disruption of the delicate balance between water supply and demand that causes a lack of enough soil moisture for plant growth. Drought is one of the main obstacles for agriculture production worldwide (Farooq *et al*, 2009) and is the most important environmental factor affecting crop yield (Boyer & Westgate, 2004).

Although UK is one of the world's most efficient producers of arable crops, 30 % of UK wheat-growing areas are prone to drought (Foulkes *et al*., 2007), thus reduce crop production. Many other countries are also severely affected by drought; for example, many states in India suffer from an average of 40 % crop loss once every five years due to severe drought, costing almost $650 million (Pandey *et al*, 2005).

Drought reduces wheat and maize production through a variety of morphological, physiological and biochemical effects (Farooq *et al*, 2009). Water stress reduces the rate of cell division, cell growth and photosynthesis in plants, causing plant damage and a reduction of crop yields (Davies & Zhang, 1991; Farooq *et al*, 2009).

Water scarcity also reduces both nutrient uptake by the roots and nutrient transport from the roots to the shoots, due to restricted transpiration and less active transport, resulting in a reduction of plant growth (Hu & Schmidhalter, 2005).

Drought can affect photosynthesis due to stomatal closure, which results from an alteration of the transpiration balance to avoid losing excess water; this causes poor plant biomass (Davies & Zhang, 1991; Farooq *et al*, 2009). Drought also reduces a plant's ability to regenerate RUBP, which causes a further decrease in photosynthesis (Nogues & Baker, 2000).Thus it is very important to investigate plant resistance to drought, and how the effects of drought can be reduced to prevent crop loss.

1.3 *Aphis gossypii* (aphid) and *Schistocerca gregaria* (locust)

A) *Aphis gossypii*

A. gossypii, superfamily Aphidoidea, are small (1-10 mm), soft bodied, phloem sucking insects. As there are approximately 4000 different species of aphids and they are one of the most harmful pests for agriculture and crops (Dixon, 1985), is important to study their effects on crop growth and production.

A. gossypii are cosmopolitan and highly polyphagous insects and they are widely distributed in tropical, subtropical and temperate regions (Satar *et al*, 2005). *A. gossypii* are generally found in clusters, preferring new, succulent shoots and new leaves. As they arephloem feeders, plant damage is directly caused by sap ingestion and potential introduction of viruses and other pathogens to phloem cells (Goussain *et al*, 2005).

Although phloem sap is rich in sugars, it is relatively poor in amino acids, thus requiring *A. gossypii* to obtain very large amounts of sap to fulfil their nutrient requirements (Dixon, 1985). An adult *A. gossypii* requires 2.1 µl of sap per day and first instar aphids require 0.8 µl of per day (Dixon & Longa, 1973). Excess sap is

excreted by aphids on the plant leaves in sticky droplets (called honeydew) (Dixon, 1985). Honeydew supports the growth of sooty moulds (a fungi), the presence of which can reduce the photosynthetic rate and can cause reduction of plant growth (Ryan *et al*., 1990; Patel & Patel 1997; Satar *et al*., 2005).

Phloem feeders such as *A. gossypii* also have a direct impact on the physiological processes of plants (Minks & Harrewijn, 1988). The effects of aphid herbivory include a reduction in leaf area, leaf curl, wilting, a reduction of shoot growth, and a delay and reduction in production of flowers and fruits (Minks & Harrewijn, 1988).

Although *A. gossypii* has many predators from a diverse range of insect families (namely Coleoptera, Hemiptera, Diptera, Neuroptera and Dermaptera), the effective biological control of aphid numbers is restricted by a need for high aphid abundance to support a high predator abundance (Sunderland, 1988; Solomon *et al*, 2000). Other biological, chemical and integrated techniques have been tested for their effectiveness at protecting agricultural crops from *A. gossypii* attack (Minks & Harrewijn, 1988). The experiment described here investigates whether silicon accumulation can protect plants from the harmful effect of aphids.

B) *Schistocerca gregaria*

S. gregaria (desert locust, family Acrididae) is perhaps the most destructive and abundant agricultural pest (Despland *et al*., 2000). The extent of crop destruction can be dependent on an interesting characteristic of locusts - density dependent polyphenism (Lovejoy *et al*., 2006; Despland *et al*., 2000). When population density is low, locusts are solitary, but at high density they are gregarious and form massive swarms. In this condition they can migrate long distances and cause massive crop destruction (Uvarov 1977; Simpson *et al*., 1999), thus in gregarious swarming state, they can cause a serious threat to agriculture (Dutta *et al*., 2001). Due to their extraordinary ability to fly rapidly

across long distances, locusts can cause massive destruction to agricultural land several hundred kilometres away from their origin (Dutta *et al*., 2001).

S. gregaria is generally found in arid and semi-arid regions of Africa, the Middle East and South West Asia (Woldewahid *et al*., 2004). Their geographical distribution ranges from 16 to 29 million km^2, encompassing almost 65 countries (Pedgley, 1983; Werf *et al*., 2005). They have caused an extreme reduction in agricultural production in Africa, the Middle East and Asia for centuries. A single female *Schistocerca* is capable of eating approximately 1.5 g of vegetation per day. However in the gregarious state, this can dramatically increase to approximately 45-225 g/m^2/day (Chapman, 1976). More than 60 countries are under threat from the destructive effects of locust swarming (Dutta *et al*., 2001). The livelihood of at least one-tenth of the world's human population can be affected by a large locust outbreak (Dutta *et al*., 2001).

.

1.4 Silicon

Silicon (Si) is the second most abundant element in the soil after oxygen. Even though silicon is not considered to be an essential nutrient for plant biology, it is recognized as a beneficial element (Epstein 1999; Fauteux *et al*., 2005; Ma & Yamaji, 2006; Gong *et al*., 2008).

Most soil-rooted plants contain a moderate amount of silica (SiO_2) (Ma & Yamaji, 2006). Silicon deposition in plants is dependent on the plant species and its developmental stage (Mecfel *et al*. 2007), and its accumulation can range between 10 and 100 g/kg of the plant dry weight (Elawad *et al*. 1979). However, plant species which contain 1 g/kg silicon on a dry weight basis are also considered as silicon accumulators. Monocots are generally better silicon accumulators than dicots. For instance, dicot crop species such as the tomato, cucumber and soybean contain less than 1 g/kg Silicon (Epstein, 1994), whereas monocot crop species such as wheat, oat, rye, barley, sorghum, maize, and sugarcane contain about 10 g/kg, and aquatic monocot crop species have a silicon concentration of up to 50 g/kg (Epstein, 1994).

Plants generally take up silicon in the form of silicic acid [$Si(OH)_4$], which is a neutral monomeric molecule (Ma & Yamaji, 2006), by diffusion and by the influence of transpiration-induced root absorption known as mass flow (Elawad *et al.* 1979). It is transferred from root to shoot via xylem and when concentrated over a critical level (approximately 100 ppm at biological pH), it polymerizes as opaline phytoliths (Reynolds *et al*., 2009). Deposition of silicon in root cell walls can help to grow a stronger root system (Epstein, 1994). In most plants, silicon is deposited within the lumen of epidermal cells, cell walls, intercellular spaces or external layers in the form of phytoliths (Emanuel Epstein, 1994; Fauteux *et al*., 2005; Massey & Hartley, 2006; Hunt *et al*., 2008). After deposition, amorphous silica becomes immovable and cannot be redistributed (Emanuel Epstein, 1994).

1.4.1 Silicon's several beneficial roles on plants

There are several hypotheses concerning the role of silicon in plants. It has been shown that silicon has a positive effect on reproduction, and the alleviation of metal toxicity and nutrient imbalance in plants (Epstein 1999; Ma & Yamaji 2006). Silicon accumulation may also potentially protect plants from a variety of biotic and abiotic stresses, such as plant pathogens, herbivores and drought (Epstein 1999; Fautex et al., 2005; Ma & Yamaji 2006; Liang *et al*., 2007).

I) Silicon accumulation as a defence against plant natural enemies

Silicon protects plants from fungal and pest attack through several mechanisms. It can increase a plant's natural resistance against pathogenic fungi by inducing immune and inflammatory responses and stress hormone production in plant cells. Silicon also can modulate the activity of post- elicitation intracellular signalling systems. This signalling system leads to the expression of defence genes, structural cell wall modification, stress hormone synthesis and antimicrobial compound synthesis (Fauteus *et al*., 2005). With

the deposited silicon beneath the cuticle, plants can make a double layer of silicon and cuticle which appears to mechanically impede penetration by viruses or fungi (Ma & Yamaji, 2006; Fauteux et al., 2005).

The most likely mechanisms of silicon action in increasing plant resistance to pest attack are reduced digestibility, and increased hardness and abrasiveness of plant tissues due to silicon deposition (Reynolds et al., 2009; Massey & Hartley., 2009; Kaufman et al., 1985), deterring both vertebrate and invertebrate pests (Hunt *et al*., 2008). Phytoliths can reduce the absorption of nitrogen from food by herbivores, which reduces their growth rates (Massey & Hartley 2006; Massey *et al*., 2006). Grasses treated with silica are more abrasive than grasses without silica treatments, and the abrasiveness of the leaves is proportional to silicon content (Hunt *et al*., 2008; Massy *et al*., 2007). Herbivores have a tendency to choose low silica plants rather than high silica plants, and they don't grow very well if they are forced to eat high silica plants (Massey and Hartley 2006).

Reynolds *et al*. (2009) discussed three main ways in which silicon may offer protection to plants against herbivores. Firstly, a band of silicon bodies might protect the underlying vascular tissue, restricting chewing herbivores to intercostal zones. Secondly, deposited silicon on epidermal cell walls might resist herbivore entry in those areas. Lastly, silicon deposition on the leaf margin might inhibit penetration.

Hunt *et al*. (2008) also suggested similar silicon defence mechanisms to protect plants against herbivores. Silicon might act chemically to protect plants from pests by reducing their digestion or absorption.

II) Silicon accumulation as a protection against drought

Silicon accumulation may also help to defend plants against abiotic stresses such as drought. Silicon can improve crop yield by aiding drought tolerance in plants incrementally through photosynthesis and a reduced transpiration rate (Gong *et al.,* 2003); it can help in thickening the leaf's specific area to reduce the transpirational loss of water (Savant *et al*., 1999; Kaya *et al.*, 2005). Silicon accumulation can also enhance calcium and potassium accumulation in the leaf, increase the relative water content, and increase the total shoot and root biomass (Kaya *et al.*, 2005). Accumulation of calcium and potassium in plant tissue is beneficial to achieve better growth in water stressed conditions (Cachorro *et al*., 1994). Silicon is reported to be an environmentally friendly alternative to the chemically based control strategy used by farmers against pathogenic bacteria, fungi, pests and in different conditions of stress (Prabhu *et al*. 2001).

1.4.2 Silicon deposition in *Triticum aestivum* and *Zea mays*

The deposition of silicon is also dependent upon the photosynthetic pathway of the plant and differs between C3 grasses such as *Triticum aestivum* and C4 grasses such as *Zea mays* (Kaufman *et al.*, 1984; Epstein, 1994). C4 plants can contain denser silica bodies in upper and lower epidermis than C3 plants (Kaufman *et al.*, 1984). In *Triticum aestivum*, silicon is generally deposited on the lower (abaxial) epidermal cells of young wheat leaves, while in old wheat leaves it deposits on upper (adaxial) epidermal cells (Epstein, 1994). Mecfel *et al*. (2007) also showed the same result: in wheat plants, deposition of silicon predominantly occurs in leaves and this concentration can increase as more silicon is added to the soil.

Mitani *et al.* (2009) identified three silicon transporter genes in maize plants: *ZmLsi*1, *ZmLsi*2 and *ZmLsi*6. The *ZmLsi*1 and *ZmLsi*6 transporters showed influx transport

activity whereas *ZmLsi*2 showed only efflux activity, similar to the rice silicon transporter (Mitani *et al.*, 2009).

As researchers and growers become more aware of silicon and its potential use in agriculture, it is likely that this often overlooked element will be recognized as a viable means of sustainably managing important plant diseases worldwide and protecting plants in several conditions of environmental stress.

1.4.3 Silicon defends against biotic stresses

I) Silicon defends against phloem feeders

Silicon plays diverse and numerous roles in protecting plants under stressful conditions (Epstein, 2009). Goussain et al (2005) showed that an elevation of silicon concentration in soil and application of a foliar silicon spray reduces the impact of pests on crops, with the longevity and reproduction rate of aphids on wheat plants shown to decrease significantly within seven days. This investigation also reported that silicon reduced the number of honeydew droplets per plant. The authors proposed that silicon could accumulate in tissue spaces, the cell wall matrix and inside cells of the plants and that the deposition of silica could increase the rigidity of cell walls, impeding the penetration of aphid stylets.

Moraes *et al.* (2005) showed that *Z. mays* plants treated with soil silicon applications followed by one or two foliar silicon applications had significantly lower numbers of aphids, whilst silicon applied only through the soil did not reduce the number of aphids. The authors proposed that following the soil application of silicon with foliar sprays might serve to create a mechanical barrier within the plant, and also stimulate some of the plant's chemical defences, the combination of which may alter the feeding preference of aphids and reduce their number.

Massey & Hartley (2006) found that aphids did not suffer any detrimental effect from increased plant silicon application via soil.

II) Silicon defenses against chewing herbivores

Massey & Hartley (2006) found that silicon application reduced the feeding and growth rates of voles by 75 % and 40 % respectively. Silicon increased leaf abrasiveness by 29-42 % and deterred vole feeding by 75 % and 63 % in *Festuca ovina* and *Lolium perenne* respectively. Excess silicon application significantly reduced vole body mass and reproductive rate. Massey *et al.* (2006) showed that soil silicon application increased leaf abrasiveness of grass species by 28-52 %. Silicon induction deterred the feeding and reduced digestion efficiency of *S. gregaria* and *Spodoptera exempta* (two folivorous insects), reducing their growth rate and altering their feeding preference towards non-silicon plants compared to silicon added plants when both were provided together (Massey & Hartley, 2006).

Silicon reduces the mechanical disruption of the cell wall of chlorenchyma (Hunt *et al* 2008). This finding suggested that less chlorophyll was released from the biomass of high silica grasses by grinding compared to control plants, with more chlorophyll detectable in the faecal matter of locusts after chewing and digestion of the control plants. Phytoliths might also strengthen chlorenchyma cells, resulting in a reduction of mechanical breakdown of cell walls by herbivores.

Epstein (2009) stated that excess silicon application provides more roughness and toughness to plants compared to control plants, thereby obstructing the penetration of cell walls by herbivores and pathogens.

III) Silicon defends against fungi and other pathogens

Foliar silicon spray and root-applied silicon could increase plant resistance to pest attack by making a physical barrier or creating natural defences in plants. Liang *et al*. (2005) showed that two cucumber cultivars treated with silicon experienced significantly reduced infections by *Podosphaera xanthii* (powdery mildew fungi) compared to control plants, and concluded that this was due to the production of a physical barrier of deposited silicon on leaf surfaces.

A similar result was achieved by Fautex *et al*., (2005), showing that silicon created a mechanical barrier to protect plants from penetration by fungi or other unwanted pathogens. Bi *et al*., (2006) also showed anti -microbial activity of silicon in Hami melons when applied to the soil, with the mycelial growth of *Fusarium semitectum* and *Trichothecium roseum* decreasing significantly with increasing silicon concentration, and a consequently reduced decay. The authors suggested that silicon has broad-spectrum anti-microbial properties, which might enhance natural fruit defences and protect fruit from decay.

Guevel *et al*. (2007, also found that root and foliar silicon application significantly reduced powdery mildew in wheat plants.

1.4.4 Silicon defends against abiotic stresses

A number of studies have shown that elevated silicon levels in plants caused by silicon incorporation in soil or by foliar spray can enhance plant resistance against abiotic stresses.

Drought increases the creation of reactive oxygen species, which can encourage oxidative damage to functional molecules of the plant. Gong *et al*. (2003) showed that drought stress significantly increased the formation of hydrogen peroxide (H_2O_2), which can be diminished by silicon application. The authors proposed that environmental stresses caused formation of reactive oxygen species, which can introduce oxidative damage to plant cells by forming H_2O_2 molecule. They also found that dry matter of wheat plants treated with silicon was significantly higher than the control plants although relative water content (RWC) did not differ.

Drought reduced the total quantity of soluble protein in plants, but silicon application increased total protein content in plants (Gong *et al*., 2003).

Gao *et al*. (2004) showed that soil-applied silicon improved the efficiency of water use in maize plants under drought threat; the effect of water stress was more damaging for the growth of shoot and root system of maize plants in silicon deficient treatments. Further, Gao *et al*. (2006) found that application of silicon to the soil of maize plants under water-stressed and non-stressed conditions significantly decreased transpiration rate from both abaxial and adaxial leaf surfaces, and also decreased conductance. They also showed that enhanced levels of silicon mainly decreased transpiration from stomatal pores rather than from the cuticular layer due to a thickening layer of deposited silica with the cellulose in the epidermal cell wall of the plant.

Kaya *et al*., (2006) found that silicon incorporation into soil significantly improved biomass, chlorophyll levels, and electrolyte leakage including calcium and potassium accumulation in leaf and root of maize plants. The authors postulated that silicon improved the water-stress tolerance in maize plants by balancing membrane permeability, by increasing the chlorophyll content and by the high accumulation of Ca and K in plant tissues (the exact mechanism is not described).

1.4.5 Conclusions

Silicon is a bioactive element, which plays diverse beneficial roles for plants under different conditions of stress. Silicon-mediated defences against biotic and abiotic stresses are based on deposition of the solid amorphous form of silicon (phytoliths) in key tissues and organs of the plants. Although much research has sought to elucidate the mechanisms of this positive contribution of silicon to plant defences, these are still under consideration. Taken together, the evidence suggests that silicon is acting as a potentiator of plant defence responses and is interacting with several key components of plant stress. Therefore there are still many aspects of the relationship between silicon and plants which require more investigation.

1.5 Thesis aims and hypotheses

Based on a literature search regarding silicon and agriculture, this thesis hypothesises that:

- The presence of excess silicon in the soil can increase drought tolerance in plants, and plant biomass by improving photosynthesis.
- Enhancement of the drought tolerance of plants by the presence of silicon depends on temperature intensity. At very high temperatures (40-45 $^{\circ}$C), silicon cannot increase drought tolerance or plant biomass.
- Soil applied silicon cannot reduce the reproduction and growth rate of aphids.
- Silicon application can increase abrasiveness of the plant leaves and thus can deter folivore feeding and can protect plants from excess damage.

The principle aim of my study is to investigate the interaction between herbivory (*Aphis gosypii* and *Schistocerca gregaria*) and water stress condition on *T. aestivum* and *Z. mays*, in the presence of silicon. The present study focused on finding answers to several questions such as:

Can enhanced silicon levels increase drought tolerance and improve biomass in *T. aestivum* and *Z. mays*, and does this effect depend on temperature intensity or growth medium of plants?

Can the presence of silicon reduce the growth rate of aphids, and will it manage to reduce the damage of *T.aestivum* and *Z.mays* by *A. gosypii* and *S. gregaria*?

These questions are addressed in the three following chapters.

Chapter 2: The effects of root applied silicon application on the growth of *Triticum aestivum* and *Zea mays* plants grown under drought conditions

2.1 Introduction

T. aestivum and *Z. mays* are two of the most important crops globally of the three hundred thousand different species of plants and are growing at about 70 % arid and semi arid areas (Gong et *al*., 2003). They are used by many countries as a staple diet and for several industrial purposes.

Severe water stress from drought is a major threat to crop production. Water stress can reduce the rate of cell division and cell growth in plants thus causing damage to the biochemistry, physiology, growth and development of the entire plant (Davies & Zhang, 1991). Water stress can also directly affect photosynthesis causing multiple constraints on various cell components (Farooq *et al.*, 2009).

This has become a very common, crucial obstacle for agriculture worldwide (Farooq *et al.*, 2009) and drought has the greatest effect of any environmental factor on crop yield loss (Boyer & Westgate, 2004). Water sources for irrigation are becoming scarce – for example due to lack of rain, rain at the wrong time of the year, increased evaporation rate in tropical and subtropical countries such as Africa and south-east Asia, and additionally from climate change such as rising temperatures in certain areas or changes in precipitation patterns. Therefore, it is essential to reduce the damaging impact of drought on crop production, and to stabilise or even increase crop production under drought conditions (Sivamani *et al*., 1999).

A possible method of reducing the negative effects of water stress on plant productivity is to apply silica to the crop, thus increasing their drought tolerance (Gong *et al.*, 2003;

Kaya *et al.*, 2005). For instance, Gong *et al.* (2003) showed that soil applied sodium silicate improved plant height, leaf area and dry mass in *T. aestivum* plants compared to plants grown without silica application under well watered conditions. Additionally, under water stressed conditions, silica applied plants showed higher relative water content (RWC), water potential and leaf area compared to those plants grown without the silica treatment. Possible physiological mechanisms behind increased water stress tolerance from silicon application include the thickening of the leaf area thus reducing loss of water through transpiration (Savant *et al.*, 1999; Kaya *et al.*, 2005), increased calcium (Ca) and potassium (K) concentrations in the plant, increased RWC and increased total biomass of both shoots and roots (Kaya *et al.*, 2005; Cachorro *et al.*, 1994; Knight *et al.*, 1997; Sangakkara *et al.*, 2001; Umar, 2002).

However, although these studies showed the beneficial effects of silicon application to plant growth, further experiments are required under different conditions to better understand these effects. In this chapter, this is investigated by manipulating the watering treatment used and the silica concentration applied in two separate experiments. These two experiments differ in their temperature intensity and their growth medium composition, which will potentially affect the amount of water available to the plants.

2.2 Chapter aims and hypotheses

2.2.1 Chapter aims

The aim of this chapter was to investigate the effects of root-applied silica solution to *T. aestivum* and *Z. mays* plant growth, when grown under control and water-stressed (drought-mimicking) conditions. The first experiment investigates the effects to the shoot biomass when grown under severe drought conditions produced by a greenhouse temperature of 45 °C and in a growth medium with a higher perlite ratio.

The second experiment investigates the effects under moderate drought conditions produced by a lower greenhouse temperature of 22 °C and a lower perlite ratio, by measuring the shoot biomass and photosynthetic rate. The second experiment also investigates whether the silicon concentration used affected the shoot biomass and photosynthetic rate compared to control plants, using a low and high silicon concentration.

2.2.2 Null hypotheses

2.2.2. I. Experiment 1

The shoot biomass of *T. aestivum* and *Z. mays,* when grown at 45 °C and with a high perlite ratio, is not significantly affected by the watering treatment or root-applied silica solution, compared to control plants.

2.2.2. II Experiment 2

1) The shoot biomass and photosynthetic rate of *T. aestivum* and *Z. may*s when grown at 22 °C and with a lower perlite ratio, is not significantly affected by either watering treatment or the silica solution concentration, compared to control plants.

2) The concentration of the silica solution applied (0.0130 mol/ L or 0.065 mol/L) is not significantly affect the shoot biomass or photosynthesis rate.

2.3 Methods

2.3.1 Experiment 1

2.3.1. I Plant growth conditions and treatments

Seeds of *T. aestivum* and *Z. mays* were obtained from Rothamsted Research, Hertfordshire in 2008. Seeds of both species were sterilized in 33 % bleach solution followed by a thorough washing with distilled water. In a temperature controlled

greenhouse (University of Sussex, UK), both species were germinated on moist filter paper in a seed propagator and kept at 25°C for three days. Seeds were then transferred to plant pots at a density of five to six seeds per pot. The pots had a diameter of 15 cm and were filled with a 9:1 perlite and compost mixture, with 2.5 g of a slow-release fertilizer (Osmocote). Once the seedlings were germinated (after four days), they were thinned to one plant per pot. For the first week, plants were watered daily to ensure that there was enough root biomass to enable healthy establishment. After this period, the plants were then subjected to one of the four treatment combinations, each treatment combination consisting of ten replicates, arranged in a fully randomized block design. The experimental design consisted of a 2-way factorial design, consisting of the treatments 'Silicon Treatment' and 'Watering Treatment'. 'Silicon Treatment' consisted of the levels 'Silica Control' and 'Silicon Application', and 'Watering Treatment' consisted of the levels 'Watering Control' and 'Drought'. These treatment combinations are described below.

1) Silica Control + Watering Control (C +W): 30 ml and 50 ml of tap water were applied on alternative days throughout the experiment. No silica was added to the plants.

2) Silica Application + Watering Control (Si + W): 30 ml of the silica solution and 50 ml of tap water were applied on alternate days throughout the experiment.

3) Silica Control + Drought (C + D): No silica solution application and only 30 ml of tap water was applied every other day to maintain drought conditions.

4) Silica Application + Drought conditions (Si + D): 30 ml of the silica solution was applied every other day to maintain drought conditions.

As the plants would not be able to survive for the experimental duration without watering, drought conditions were produced by watering the plants with a small amount of water on alternative days, and all the plants were grown in the same greenhouse, maintained at 40-45 °C and 40-50 % humidity. Silicon was applied to the roots of the plant as a silica solution. 0.0065 mol/ L silica solution was made using 0.65 g of

$NaSiO_3.9H_2O$ (Sodium metasilicate non- hydrate), per litre of distilled water. All treatments were applied to the soil surface and allowed to drain through the soil. Plants were placed on individual saucers to prevent loss of the applied water and/or silicon treatment. Plants were harvested after 50 days of growth in their treatment. Shoots and roots were separated using scissors and shoot fresh weights (FW) were recorded for each plant. Plant material was dried at 80 $^{\circ}$C and subsequently the shoot dry weight (DW) measured.

2.3.1. II Data analysis

As data were found to be normally distributed and have equal variances, the shoot biomass (DW) data were analyzed using a 2-way ANOVA, consisting of the factors 'Silicon' (levels either Control or Silica Applied) and Watering Treatment (levels either Watering Control or Drought). Post-hoc Tukey tests were used to identify the location of significant differences between group means.

2.3.2 Experiment 2

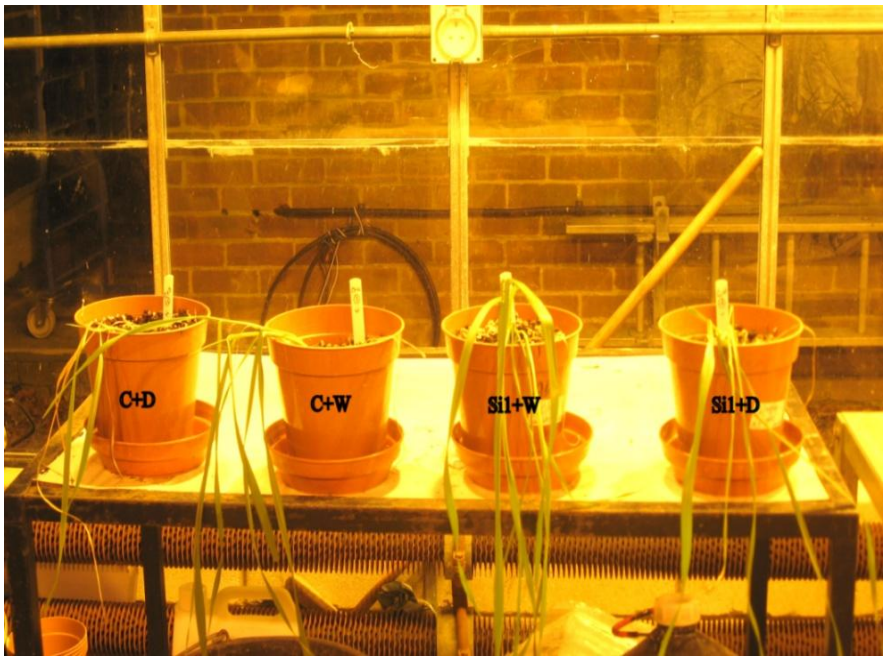
2.3.2 .I Plant growth conditions and treatments

The second experiment utilized the same methods and the same experimental design as experiment 1, with the exception that the plants were grown in a growth medium containing a 1:1:1 perlite, soil and compost mix, and the greenhouse temperature was reduced to a constant temperature of 20 -22 ℃, with a constant humidity of 40-45 %. The same method to produce the silica solution was also used but the silica solution was applied at three different concentrations: 'Control' (no $NaSiO_3.9H_2O$ (i.e. only distilled water)), 'Si 1' (0.0065 mol/L of silica solution made up with 650g of $NaSiO_3.9H_2O$ dissolved in 10 L of distilled water), and 'Si 2' (0.0130 mol/L of silica solution made up with 1300 g of $NaSiO_3.9H_2O$ dissolved in 10 L of distilled water). Again a 2-way fully factorial design was used, consisting of the treatments 'Silicon Treatment' and 'Watering Treatment'. 'Silicon Treatment' consisted of the levels 'Silica Control', 'Si 1' and 'Si 2'. 'Watering Treatment' consisted of the levels 'Watering Control' and

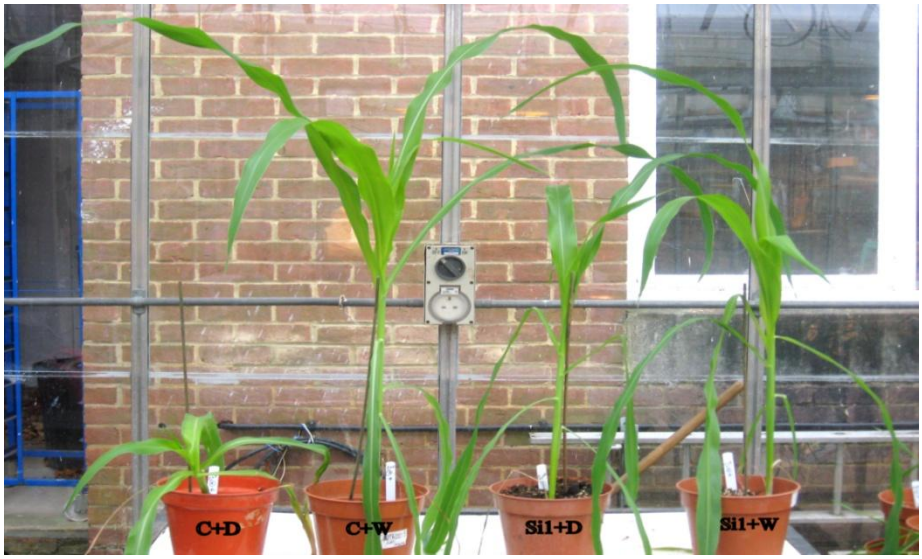
'Drought'. The treatment combination methods were the same as experiment 1, with the inclusion of the higher 0.013 mol/ L silicon solution concentration. To measure the photosynthetic rate of *T. aestivum* and *Z. mays,* a CIRAS II instrument was used on clear and sunny days. As in experiment 1, the plants were harvested after 50 days using the same harvesting technique. Photos of the experimental methods are shown in Figure 2.1.

a)

b)



C)



d)

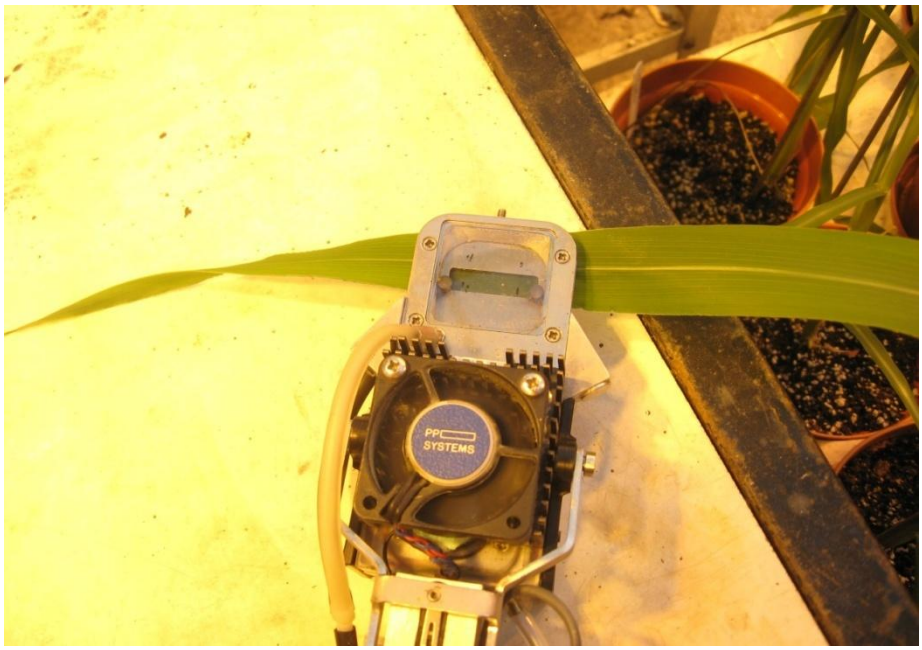


Figure 2.1 a) 4 day old *T.aestivum* and *Z.mays* seedlings, **b) and C)** *T.aestivum* and *Z.mays* plants grown under experimental drought conditions at 22 ^{o}C respectively, and **d)** Photosynthetic rate measurement of *T.aestivum* by CIRAS II.

2.3.2. II Data analysis

As data were found to be normally distributed and have equal variances, the shoot biomass (DW) data were analyzed using a 2-way ANOVA, consisting of the factors 'Silicon Treatment' and 'Watering Treatment' and their relevant levels. Post-hoc Tukey tests were used to identify significant differences between group means.

2.4 Results

2.4.1 Experiment 1

2.4.1. I Shoot biomass (DW)

A) *Triticum aestivum*

The dry weight of *T. aestivum* plants was significantly reduced by the experimental drought conditions used (Table 2.1). The shoot biomass of the drought treatment plants was reduced by 60 % overall (mean shoot biomass = 0.35 g), compared to that of the plants under the control watering treatment (mean shoot biomass = 1.00 g) (Figure 2.1). However, the application of silica solution did not affect the shoot biomass of the plants, regardless of the watering treatment used, and there was no significant interaction seen between silica application or watering treatment (Table 2.1).

*Table*2.1: Statistical output of a 2-way ANOVA on the shoot biomass of *T. aestivum*.

Source	**DF**	**SeqSS**	**AdjSS**	**Adj MS**	**F**	**P**
Silica Treatment	1	0.02	0.02	0.02	0.02	0.893
Watering Treatment	1	424.80	424.80	424.80	362.11	<0.001
Silica Treatment * Watering Treatment	1	3.79	3.79	3.79	3.23	0.078
Error	56	65.70	65.70	1.17		

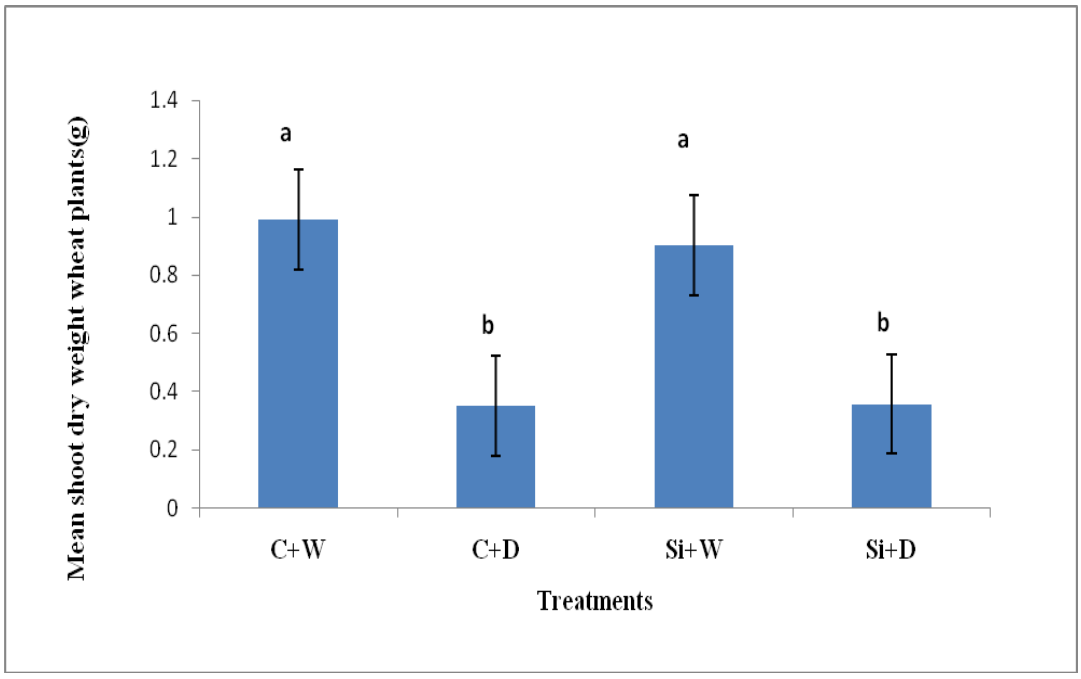


Fig 2.1: Mean (± SE mean) shoot dry weight of *T. aestivum* plants in response to watering treatment and silicon application treatment. 'C+W' =Silica Control + Watering Control, 'C+D' = Silica Control + Drought, 'Si+W'= Silica Application + Watering Control, 'Si+D' = Silica Application + Drought conditions.

B) *Zea mays*

The same trends were also observed for the shoot DW of *Z. mays* plants. Overall, the mean shoot biomass was significantly reduced by 67 % under the experimental drought conditions applied, compared to the watering treatment control plants (mean shoot biomass = 16.00 g) ($F_{1, 56}$=304.16, P<0.001(Table 2.2 and Figure 2.2). The watering treatment control maize plants reached an average biomass of 16.00 g, while the drought plants only reached an average of 5.00 g (Figure 2.2). As before, silicon application did not have a significant effect on the shoot DW biomass, regardless of the watering treatment, and there was no interaction seen between silicon treatment or watering treatment ($F_{1, 56}$=0.01, P=0.906, Table 2.2).

Table 2.2: Statistical output of a 2-way ANOVA on the shoot biomass of *Z. mays.*

Source	**DF**	**SeqSS**	**Adj SS**	**AdjMS**	**F**	**P**
Silica Treatment	1	0.09	0.09	0.09	0.01	0.906
Watering Treatment	1	1997.80	1997.80	1997.80	304.16	<0.001
Silica Treatment * Watering Treatment	1	1.42	1.42	1.42	0.22	0.643
Error	56	367.82	367.82	6.57		

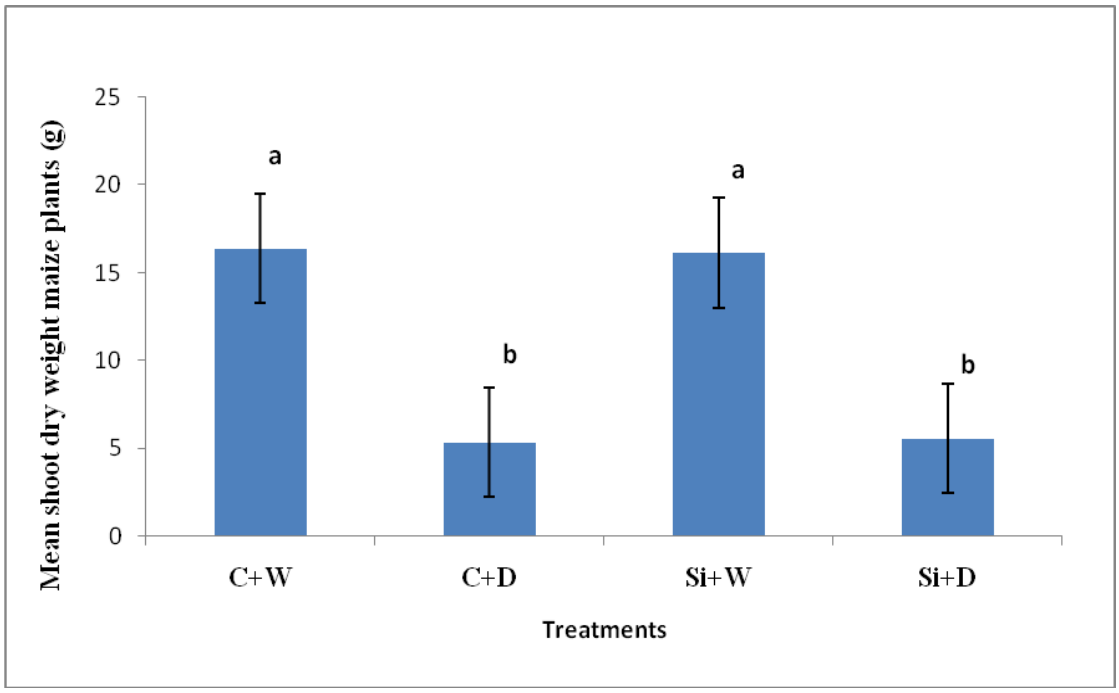


Fig 2.2 Mean (± SE mean) shoot dry weight of *Z. mays* plants in response to watering treatment and silicon application treatment. 'C+W' =Silica Control + Watering Control, 'C+D' = Silica Control + Drought, 'Si+W'= Silica Application + Watering Control, 'Si+D' = Silica Application + Drought conditions.

The results of experiment 1clearly show that the watering treatment had a significant effect on the shoot biomass of both *T. aestivum* and *Z. mays* plants, and that the drought conditions produced had a detrimental effect on shoot biomass. However, the application of silica at a concentration of 0.0065 mol/ L every other day, did not have a significant effect on the shoot biomass of both species compared to the silica treatment control plants, regardless of the watering treatment used, when grown under these experimental conditions.

2.4.2 Experiment 2

2.4.2. I Shoot biomass (DW)

A) *Triticum aestivum*

As in experiment 1, the shoot biomass (DW) of *T. aestivum* was significantly affected by the watering treatment used when grown at 20 – 22 °C; ($F_{1, 54}$=34.94, P<0.001, Table 2.1a). When grown without silica application, the shoot biomass of plants grown under drought conditions was reduced by 57 % (mean shoot biomass = 0.30 g) compared to the watering treatment control plants (mean shoot biomass = 0.70 g) (Figure 2.3). In contrast to experiment 1, when the greenhouse temperature was reduced from 40 °C to 25 °C, the soil- application of silica had a significant effect on the shoot biomass of *T. aestivum* and also significantly interacted with the watering treatment used (Table 2. 3). When *T. aestivum* was grown under the control watering treatment, the application of silica did not affect the shoot biomass, regardless of the silica concentration used. However under the drought condition the application of silica significantly increased the shoot biomass by approximately 50 %, compared to the silica applied control plants. This biomass increase produced under drought conditions was achieved by the low silicon concentration treatment (0.0065 mol/ L). However, the high Si concentration treatment used (0.0130 mol/ L) did not produce a significantly higher shoot biomass compared to the low silicon concentration (Figure 2.3).

Table 2.3: Statistical output of a 2-way ANOVA on the shoot biomass of *T. aestivum*.

Source	**DF**	**SS**	**MS**	**F**	**P**
Silica Treatment	2	0.28756	0.143780	6.90	0.002
Watering Treatment	1	0.72820	0.728202	34.94	0.000
Interaction	2	0.24005	0.120027	5.76	0.005
Error	54	1.12551	0.020843		

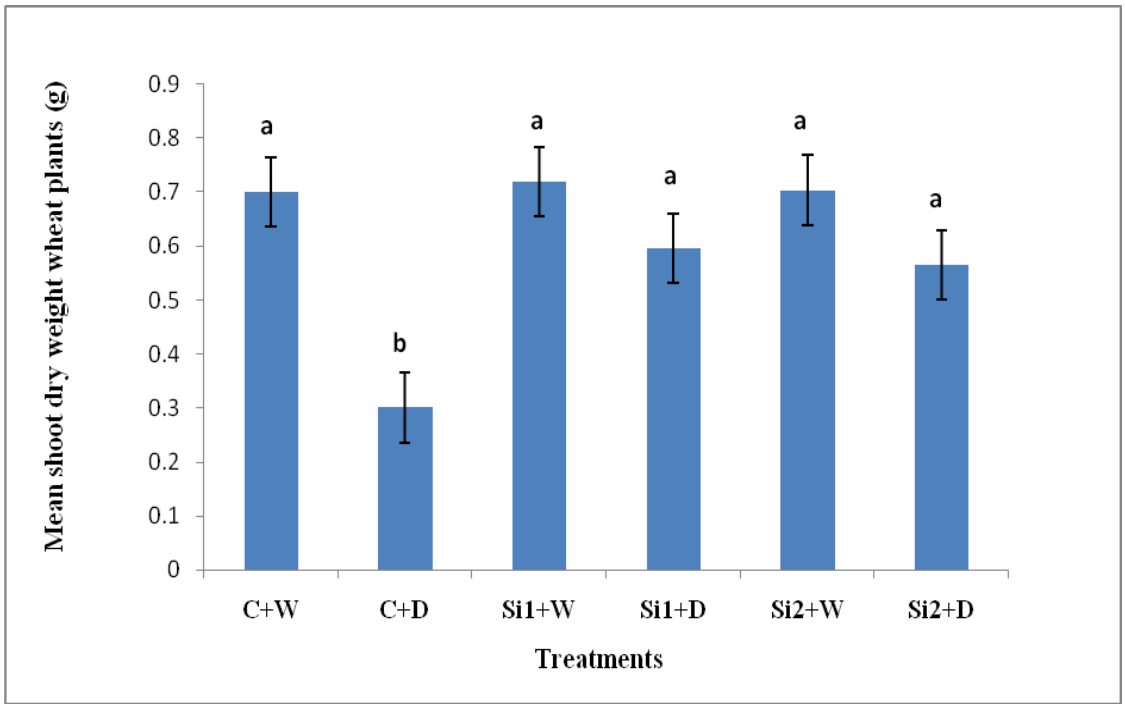


Fig 2.3: Mean (± SE mean) shoot dry weight of *T. aestivum* plants in response to watering treatment and silicon application treatment. 'C+W' =Silica Control + Watering Control, 'C+D' = Silica Control + Drought, 'Si+W'= Silica Application + Watering Control, 'Si+D' = Silica Application + Drought conditions. Si1=Low silica concentration, Si2= High silica concentration.

B) *Zea mays*

Very similar trends to the *T. aestivum* plants were also observed for *Z. mays.* Again the shoot biomass (DW) was significantly reduced when plants were grown under drought conditions at 20 – 22 °C, and this was significantly affected by and interacted with the root application of silica to the *Z. mays* plants (Figure 2.4 and Table 2.4). When plants were grown under the control watering treatment without the addition of silica solution, the mean shoot biomass was 5.0 g, and this weight did not differ on the application of silica solution, regardless of the concentrations used (Figure 2.4). However, when grown under drought conditions, the shoot biomass was significantly reduced by approximately 65 % to 1.7 g, ($F_{1, 54}$=79.30, P<0.001, Table 2.2a). The shoot biomass of

drought stressed plants was reduced by 65 % compared to that of well watered plants (Figure 2.2a) and was also significantly affected by the application of silica and significantly interacted with the watering treatment used (Table 2.4 and Figure 2. 4). Under the drought conditions, the application of silica significantly increased the shoot biomass by approximately 50 %, compared to the silica application control plants. This biomass increase produced under drought conditions was achieved by the low silica concentration treatment (0.0065 mol/ L); the higher silica concentration (0.0130 mol/ L) did not produce a significantly higher shoot biomass (Figure 2.4).

Table 2.4: Statistical output of a 2-way ANOVA on the shoot biomass of *Z. mays*.

Source	**DF**	**SS**	**MS**	**F**	**P**
Silica treatment	2	8.220	4.1100	4.92	0.011
Watering treatment	1	66.297	66.2971	79.30	0.000
Interaction	2	9.164	4.5819	5.48	0.007
Error	54	45.145	0.8360		

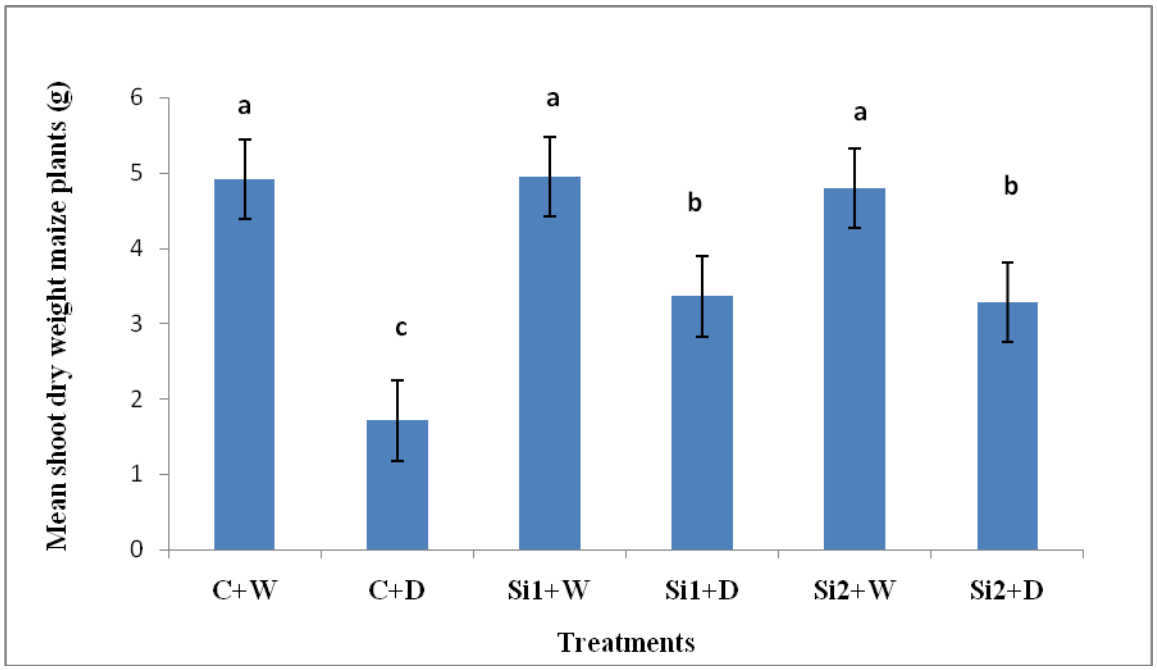


Fig 2.4: Mean (± SE mean) shoot dry weight of *Z. mays* plants in response to watering treatment and silicon application treatment. 'C+W' =Silica Control + Watering Control, 'C+D' = Silica Control + Drought, 'Si+W'= Silica Application + Watering Control, 'Si+D' = Silica Application + Drought conditions. Si1=Low silica concentration, Si2= High silica concentration.

2.4.2. II Photosynthesis rate

A) *Triticum aestivum*

The photosynthesis rate of *T. aestivum* was significantly affected by and interacted with the watering treatment and silica application treatment used (Table 2.5).When no silica solution was added to the *T. aestivum*, the drought conditions significantly reduced the photosynthesis rate of *T. aestivum;* however the application of silica solution to the drought treatment plants significantly increased their photosynthesis rate by 26 % (Figure 2.5). The higher silica concentration (0.0130 mol/ L) did not produce a significantly higher photosynthesis rate compared to the plants grown under the lower concentration of silica (Figure 2.5).

Table 2.5: Statistical output of a 2-way ANOVA on the photosynthesis rate of *T. aestivum*.

Source	DF	SS	MS	F	P
Watering Treatment	1	39.204	39.2042	264.46	<0.001
Silica Treatment	2	73.567	36.7835	248.13	<0.001
Interaction	2	33.420	16.7102	112.72	<0.001
Error	54	8.005	0.1482		

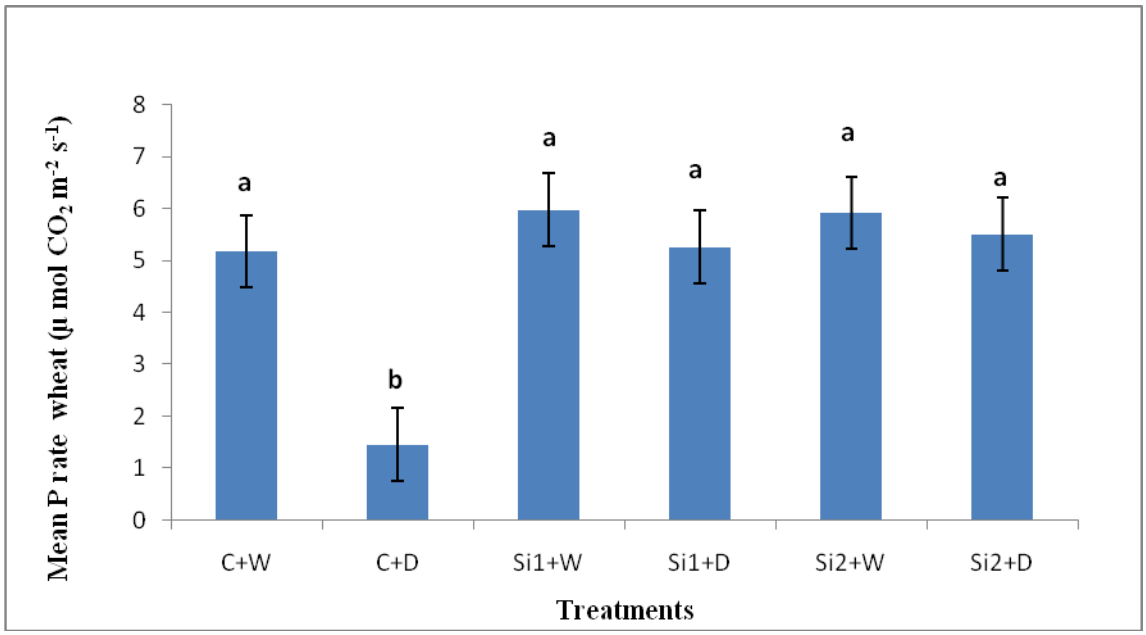


Fig 2.5: Mean (± SE mean) photosynthesis rate of *T. aestivum* plants in response to silicon treatment and watering treatment. . 'C+W' =Silica Control + Watering Control, 'C+D' = Silica Control + Drought, 'Si+W'= Silica Application + Watering Control, 'Si+D' = Silica Application + Drought conditions. Si1=Low silica concentration, Si2= High silica concentration.

B) *Zea mays*

Like *T. aestivum*, the watering treatment and silica treatment significantly affected the photosynthetic rate of *Z. mays*, again producing a significant interaction between the two experimental treatments (Table 2.6). Under the control watering treatment, photosynthesis was approximately 10 µmol CO_2 m^{-2} s^{-1}, regardless of the silicon application treatment. The photosynthesis rate of maize plants was significantly reduced by the experimental drought conditions compared to the normal watering treatment, when no silica was added to the plants. However the photosynthesis rate of *Z. mays* plants under drought conditions was significantly increased by approximately 36 % with the application of silica solution (Figure 2.6). Control plants with watering treatment produced the optimal photosynthesis rate (Fig 2.6). Like *T. aestivum*, the higher silica concentration (0.0130 mol/ L) did not produce a significantly higher photosynthesis rate compared to the lower concentration of silica (Figure 2.6).

Table 2.6: Statistical output of a 2-way ANOVA on the photosynthesis rate of *Z. mays*.

Source	**DF**	**SS**	**MS**	**F**	**P**
Watering Treatment	1	101.660	101.660	102.71	<0.001
Silica Treatment	2	237.681	118.841	120.07	<0.001
Interaction	2	322.886	161.443	163.11	<0.001
Error	54	53.449	0.990		

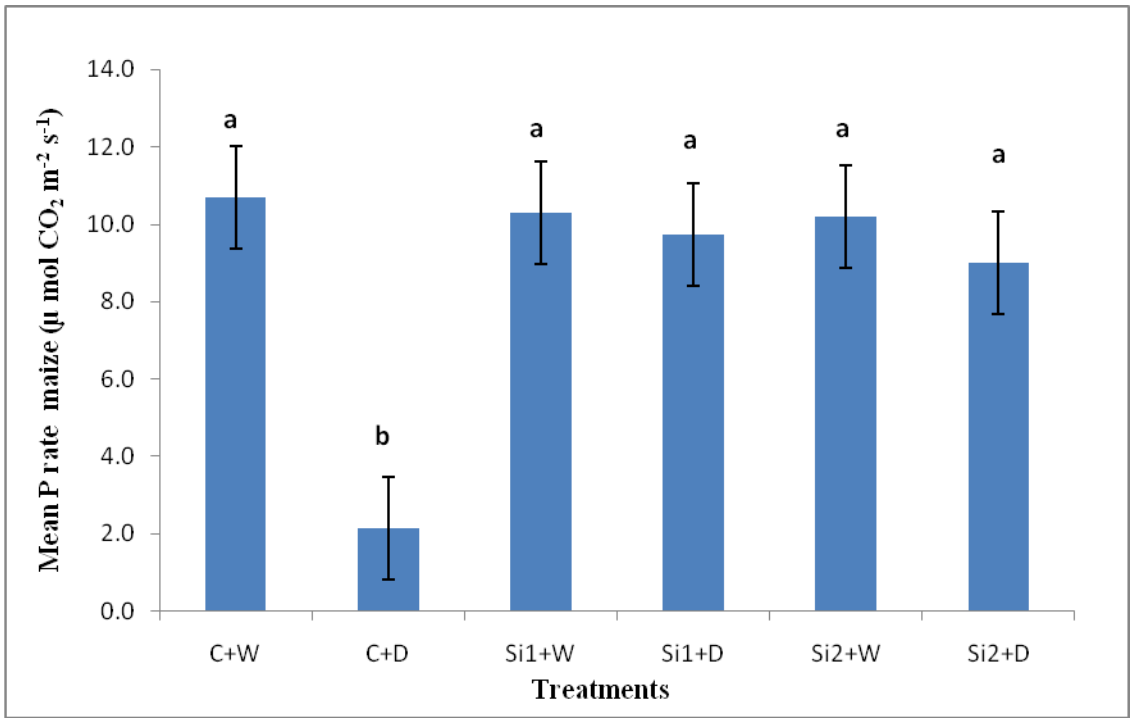


Fig 2.6: Mean (± SE mean) photosynthesis rate of *Z. mays* in response to silicon treatment and watering treatment. 'C+W' =Silica Control + Watering Control, 'C+D' = Silica Control + Drought, 'Si+W'= Silica Application + Watering Control, 'Si+D' = Silica Application + Drought conditions. Si1=Low silica concentration, Si2= High silica concentration.

In summary, the results of these experiments clearly showed that drought conditions were produced through the high greenhouse temperatures and the reduced watering frequency. Reduced growth under these drought conditions had a significant detrimental effect on the above ground biomass of both *T. aestivum* and *Z. mays* plants. The application of silica solution can help to remediate the negative effects of drought conditions; however this was found to be significant in experiment 2 but not experiment 1, and could be potentially due to the different experimental conditions used. However the higher concentrations of the silica solution used do not seem to affect the shoot growth, when the concentration applied is at least 0.0065 mol/L every other day.

2.5 Discussion

This chapter investigated the effects of root-applied silica solution on the shoot biomass and the photosynthesis rate of *T. aestivum* and *Z. mays* plants when grown under control and reduced watering treatments. Both experiments presented in this chapter clearly show that the experimental conditions used to produce drought conditions reduced the shoot biomass and photosynthesis rate of *T. aestivum* and *Z. mays* plants compared to the normal water treatment at both 22 °C (Experiment 2) and 45 °C (Experiment 1). However the effects of silica application on plant growth seem to be dependent upon the greenhouse experimental conditions used. In Experiment 1, silica solution applied to the plants (grown at 45 °C and with a higher perlite ratio) did not significantly improve the growth of both plant species under drought conditions, whereas in experiment 2 silica application did improve plant growth (grown at 22 °C and with a lower perlite ratio), under drought conditions. Although this could be due to other, unidentified experimental differences, and needs future experiments to determine the exact cause, the effects of silica application on shoot biomass may be dependent upon the water availability.

Experiment 1 used a very high and constant greenhouse temperature of 40-45^{o}C, which could have created severe drought conditions, possibly resulting in a lower water availability from increased soil evaporation, thus potentially reducing water and silica uptake by the plants. Additionally experiment 1 used a much higher ratio of perlite to compost than experiment 2 which may have also reduced the water availability to the plants. Perlite is a very light growth medium and unlike sand or soil, it might not be able to hold as much water as soil and compost, thus reducing the water capacity of the growth medium in experiment 1 compared to experiment 2. Previous studies conducted at 20-25 ^{o}C and using a 1:1:1 perlite, soil and compost growth medium (i.e. under experimental conditions similar to experiment 2), support the hypothesis that silica application assists plant growth under drought conditions, for *T. aestivum* (Gong *et al.* 2003), *Z. mays* (Kaya *et al.* 2006) and *Sorghum bicolor* (Lux *et al.* 2002) plants. Hence, the two experiments presented in this chapter suggest that the effect of silica

application as a barrier to the negative effects of water stress is dependent on both the growth medium used and the temperature to which they are exposed. Thus, silica application may be beneficial to plants grown under moderately reduced water availability, but not under severely reduced water availability.

The beneficial effects of silica application to the roots, as demonstrated by experiment 2, may be due to a variety of physiological mechanisms. For instance, Kaya *et al.*(2006) showed that root silicon application could increase the dry weight of *Z. Mays* shoots under water stressed conditions. They proposed that silica deposition in plant tissues helps to alleviate water stressed plants by decreasing transpiration and improving light interception by keeping the leaf blade erect. They also stated that the thick layer of silica gel associated with the cellulose in the epidermal cell walls might also help reduce water loss.

Gong *et al.* (2003) showed that application of excess silica solution to *T. aestivum* plants under drought and well-watered conditions significantly increased their dry weight, although plant height was not affected by silica application. They showed that root applied silica solution increased the growth of *T. aestivum* plants predominantly through increased cell elongation or cell division and produced a larger leaf area in plants under drought conditions. Agarie *et al*. (1998) found that the cuticular transpiration rate of *Oryza sativa* (rice) plants was reduced by 35 % in the presence of silica application. In contrast, Gao *et al.* (2006) showed that silicon application significantly decreased transpiration rates and conductance of water in the leaves of *Z. mays* plants grown under water stressed and non-water stressed conditions. Gao *et al*. (2006) suggested that the different trends for these two plant species, produced by the application of silica, was due to the greater accumulation of silica bodies by *Oryza sativa* plants compared to *Z. mays* plants. As Gao *et al*. (2006) did not find any silica deposition on the stomata of either abaxial or adaxial surfaces of *Z. mays* plants for either silicon treatment, nor were the changes in stomatal density or structure directly affected by and related to silicon application, they proposed that silica application

might influence stomatal opening and consequently reduce transpiration rate. However, the complete physiological mechanisms behind this are unknown.

Experiment 2 supported the results of Gao *et al.* (2006), as root-application of silica solution to water-stressed *T. aestivum* and *Z. mays* plants significantly increased the photosynthetic rate of water-stressed plants, leading to the improvement of plant shoot biomass to levels equal to or higher than the watering treatment control plants, thus dramatically reducing the impact of water-stress under these experimental conditions on the growth of the plants.

In experiment 2, the higher silica concentration (0.0130 mol/ L) did not have any extra significant effects on the shoot biomass and on the photosynthesis rate of *T. aestivum* and *Z. mays* compared to the lower silica concentration (0.065 mol/ L). One suggestion for this observation is that 0.0130 mol/ L silica concentration may not be strong enough to show greater beneficial effects in plants compared to the 0.065 mol/ L silica solution; alternatively, there may be a certain limit of silica uptake by plants from the soil. This needs further research.

2.6 Conclusion

This chapter demonstrates that silica application may increase plant biomass under drought conditions, but the extent of this is potentially dependent on the growing temperature and the water availability of the growth medium used. Thus, root-applied silica solution may not improve plant biomass under extreme drought conditions. The temperature-dependent effects of silica application do not seem to have been investigated in earlier studies, so the experiments presented in this chapter suggest that although silica application to plants under drought conditions might be an effective pathway to improve plant biomass under drought conditions, the temperature-dependency of this requires further investigation. Higher concentrations of silica were

not shown to produce greater beneficial effects on biomass and photosynthesis in this experiment; however the concentration increase used here may not have been high enough to show an effect. A further possible limitation of this experiment is that the growth medium may also affect the water availability and consequently the silica uptake of *T. aestivum* and *Z. mays* plants.

Chapter 3: The effects of silica application on *Triticum aestivum* and *Zea mays* plant growth and palatability when grown under water stress and *Schistocerca gregaria* herbivory

3.1 Introduction

S. gregaria (desert locust, Family Acrididae) is one of the most notorious agricultural pests (Desplan*et al*., 2000). *S.gregaria* has a wide geographic distribution from West Africa to India, and extending north to Iran and south to Kenya (Chapman, 1976). When the population density is high, *S. gregaria* displays gregarious behaviour and forms massive swarms, migrating long distances in search of food (Uvarov 1977; Pener 1991; Simpson *et al*., 1999).

Juvenile *S.gregaria* can occur in swarms with densities of 100 to 1000 individuals per m^2. Adult swarms can extend over 100km and a single swarm may contain more than 10^9 *S.gregaria,* weighing around 1,500,000 kg. These insects are capable of eating approximately their own weight vegetation daily, so they can cause an immense amount of damage to crops (Chapman, 1976).

As discussed in the general introduction silicon has been found to protect plants from different biotic and abiotic stresses including pest attacks (Epstein 1999; Fautex *et al*., 2005; Ma &Yamaji 2006; Liang *et al*., 2007). It has been shown that plants can actively uptake silica from soil in the form of silicic acid. The majority of silica taken up by plants from the soil is deposited as hydrated amorphous silica within the lumen of epidermal cells, forming solid silica bodies called phytoliths (Ma &Yamaji, 2006; Kaufmann *et al*., 1985; Epstein, 1994; Fauteux *et al*., 2005; Massey & Hartley, 2006; Hunt *et al*., 2008).

Phytoliths can increase the structural rigidity of plants and can protect plants from pest attacks (Epstein *et al.*, 1994; Belanger *et al.*, 1995; Savant *et al.*, 1999), for example by increasing the abrasiveness of the leaves of grass species, consequently wearing down the teeth of chewing herbivores and deterring feeding, creating a mechanical barrier (Massey & Hartley, 2006; Hunt *et al.*, 2008). Furthermore, phytoliths can also reduce the nitrogen absorption from food by herbivores, with a negative influence on their growth rate (Massey & Hartley 2006; Massey *et al.*, 2006).

Massey *et al.* (2006) showed that the presence of silicon in various grass species deterred herbivory and reduced the digestion efficiency of two generalist folivore insect species – *S. exempta* larvae and *S. gregaria*- thus reducing their growth rate. The relative growth rate of *S.gregaria* nymphs was reduced by 17-33 % on high silica grasses, compared to low silica grasses. When forced to feed on high silica grasses, the efficiency by which S.*gregaria* nymphs were able to convert leaf mass to body mass was reduced by 50-70 % for three different grass species.

Therefore, silicon application to crop plants may provide a method of reducing damage inflicted by chewing herbivores such as *S. gregaria.* Additionally most *S. gregaria* outbreaks have been recorded during drought conditions (White, 1975), and they originate mainly in warmer regions (Chapman, 1976). Therefore the influence of silica application on herbivory to plants under drought conditions is important in assessing the potential of silica application to reduce *S. gregaria* herbivory.

3.2 Chapter aims and hypotheses

3.2.1 Chapter aims

The experiment presented in this chapter aimed to investigate the effect of silica solution application on shoot biomass of *T. aestivum and Z. mays* plant species, under drought conditions and in the presence of *S. gregaria*, and analyses how these factors may interact with each other. This experiment also measured the percentage damage inflicted on the plants in the presence of *S. gregaria*, and how this was affected by the watering and silica treatments.

3.2.2 Null hypotheses

1) The shoot biomass of *T. aestivum* and *Z. mays* is not significantly affected by the watering treatment or silica treatment.
2) The percentage plant damage by *S. gregaria* is not significantly affected by the watering treatment or silica treatment.

3.3 Methods

3.3.1. Plant growth conditions and treatments

T. aestivum and *Z. mays* plants were grown as described in Chapter 2, Experiment 1.

Once plants were germinated they were thinned to one plant per pot. For the first week, plants were watered daily to ensure that there was enough root biomass to enable healthy establishment. After this period (1 week), they were subjected to one of eight treatment combinations, each consisting of ten replicates, arranged in a fully randomized block design. The experimental design consisted of a 3-way factorial design, consisting of the treatments ‘Silica Treatment’, ‘Watering Treatment’ and ‘Locust Treatment’. ‘Silica Treatment’ consisted of the levels ‘Silica Control’ (-Si) and ‘Silicon Application’ (+Si); ‘Watering Treatment’ consisted of the levels ‘Watering Control’ (W) and ‘Drought’ (D); and ‘Locust

Treatment' consisted of the levels 'Locust Control' (-L) and 'Locust added' (+L). These treatment combinations are described below in Table 3.1.

Table 3.1: Design of Experiment 1. 'Silica Treatment' consisted of the levels 'Silica Control' (-Si) and Silicon Application' (+Si); 'Watering Treatment' consisted of the levels 'Watering Control' (W) and 'Drought' (D), and 'Locust Treatment' consisted of the levels 'Locust Control' (-L) and 'Locust added' (+L).

Treatment Combination		**Treatment Combination Levels**		
No.	**Code**	**Silica Treatment**	**Watering Treatment**	**Locust Treatment**
1	C+W	-Si	W	-L
2	C+D	-Si	D	-L
3	C+W+L	-Si	W	+L
4	C+D+L	-Si	D	+L
5	Si+W	+Si	W	-L
6	Si+D	+Si	D	-L
7	Si+W+L	+Si	W	+L
8	Si+D+L	+Si	D	+L

Silicon was applied to the roots of the plant as a 0.0065 mol/L solution, as described in chapter 2. For the silica treatment levels, +Si plants received 30 ml of silica solution every other day, and –Si plants (controls) received 30 ml of tap water every other day. Watering and drought conditions were maintained as described in chapter 2.The greenhouse temperature was kept at 42-45°C with 40-50% humidity during the course of the experiment. Photos of the experimental plants are shown in Figures 3.1 **a)** and **b)** for *T. aestivum* and *Z. mays* respectively.

a)

b)

Figure 3.1: Caged **a)** *T. aestivum* plants and **b)** *Z. mays* plants grown under experimental drought conditions and with *S. gregaria* present.

3.3.2 *Schistocerca gregaria* culture and treatment

Second instars of *S. gregaria* (Figure 3.2) were obtained from a local pet shop, housed in glass cages and maintained on a diet of grass and water before use. Plants were grown for 45 days under their watering and silica treatments before *S. gregaria* were added to ensure that the plants had established enough biomass for the herbivores to consume. Two locusts were added per plant in the 'Locust added' treatment group (+L). All plants in the experiment (including the Locust control plants (-L)), were covered by 90 cm tall muslin cages under the greenhouse environment with 12 hours full light penetration. Plants were left for a further 15 days before harvesting, to ensure that the locusts had opportunity to consume the leaves.

Figure 3.2: 2nd instar *S. gregaria.*

3.3.3 Shoot damage

The overall % damage per plant was calculated using the following formula:

$$\text{Overall \% damage per plant} = \frac{\text{Average \% damage per leaf x Number of damaged leaves}}{\text{Total number of leaves on plant}}$$

3.3.4 Plant harvesting

Plants were harvested after 60 days of growth in their treatment; fresh and dry weights were recorded as described in chapter 2.

3.3.5 Data analysis

For both plant species, the shoot biomass (DW) data and plant damage % data were found to be normally distributed and to have equal variances. Therefore, data for each species were analyzed using a general linear model (GLM), consisting of the factors 'Silicon' (levels either Control or Silica Applied), Watering Treatment (levels either Watering Control or Drought) and Locust Treatment (with or without *S. gregaria*). Post-hoc Tukey tests were used to identify significant differences between group means.

3.4 Results

3.4.1 Shoot biomass

A) *Triticum aestivum*

The results showed that the shoot dry weight of *T. aestivum* plants grown at 40-45 °C was significantly affected by the watering treatment, but the locust treatment and the application of silica solution did not have a significant effect, nor were there any significant interactions between the treatments (Table 3.2).The shoot biomass of plants under drought conditions at 40-45 °C was significantly reduced by about 50 % compared to the watering treatment control plants (Figure 3.3). However, the impact of drought conditions on the shoot biomass of water stressed plants did not significantly interact with the silica application treatment. The presence of *S. gregaria* did not significantly reduce the shoot biomass after 15 days, and this did not significantly interact with the application of silica solution (Table 3.2 and Figure 3.3).

Table 3.2: GLM statistical output for shoot biomass (DW) of *T. aestivum* plants.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Silica treatment	1	0.850	0.850	0.850	0.43	0.512
Watering treatment	1	67.649	67.649	67.649	34.52	0.000
Locust treatment	1	1.793	1.793	1.793	0.91	0.341
Silica treatment *						
Watering treatment	1	1.799	1.799	1.799	0.92	0.340
Silica treatment*						
Locust treatment	1	0.130	0.130	0.130	0.07	0.797
Watering treatment*						
Locust treatment	1	0.938	0.938	0.938	0.48	0.491
Error	113	221.424	221.424	1.960		

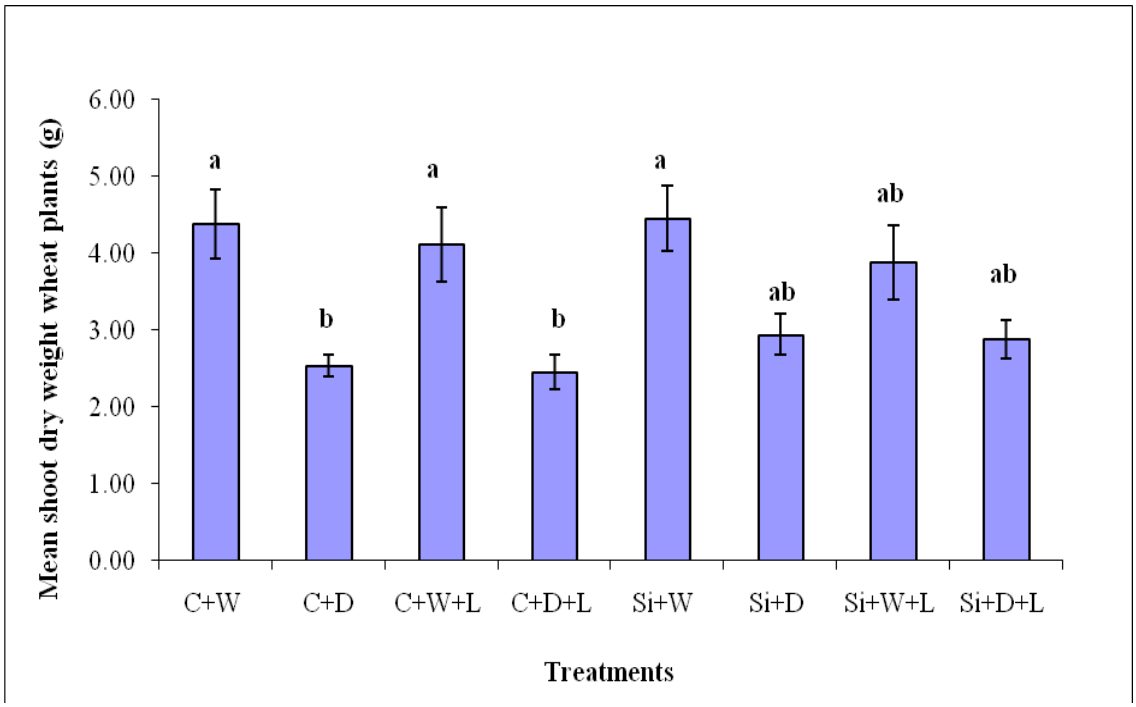


Figure 3.3: Mean (± SE mean) shoot dry weight of *T. aestivum* plants in response to silica application treatment, watering treatment, and locust treatment. C = Silica treatment control, Si = Silica solution applied. W = Watering treatment control, D = Drought conditions. L = *S. gregaria* locusts present.

B) *Zea mays*

The shoot dry weight of *Z. mays* plants grown at 45 °C was also significantly affected by the watering treatment and additionally the locust treatment; however the application of silica solution did not have a significant effect on shoot biomass, nor were there any significant interactions between the treatments (Table 3.3). As with *T. aestivum*, silica application did not have any significant effect on reducing the impact of drought on plant biomass of *Z. mays* plants. However, the presence of locusts did have a significant effect on *Z. mays* plant biomass, which was not observed in *T. aestivum* plants. The above ground biomass of *Z. mays* was significantly reduced by drought conditions imposed on the plants. When grown without the silica solution

application, the watering treatment control plants reached an average of 6.20 g of shoot biomass, whilst the water-stressed plants reached an average of 4.90 g of biomass (Figure 3.4). However, when grown with the silica solution application, the watering control plants and the water stressed plants obtained an average of 5.90 g and 4.80 g of shoot biomass respectively. Additionally for the silica application control plants, the presence of *S. gregaria* reduced shoot biomass by an average of 0.60 g in the watering treatment control plants and by 0.80 g in the water stressed plants (Figure 3.4).

Table 3.3: GLM statistical output for shoot biomass (DW) of *Z. mays* plants.

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Silica treatment	1	1.230	1.230	1.230	1.16	0.284
Watering treatment	1	32.329	32.329	32.329	30.58	0.000
Locusts treatment	1	10.058	10.058	10.058	9.51	0.003
Silica treatment*						
Watering treatment	1	0.351	0.351	0.351	0.33	0.566
Silica treatment*						
Locusts treatment	1	0.000	0.000	0.000	0.00	0.984
Watering treatment*						
Locusts treatment	1	0.067	0.067	0.067	0.06	0.802
Error	72	76.127	76.127	1.057		

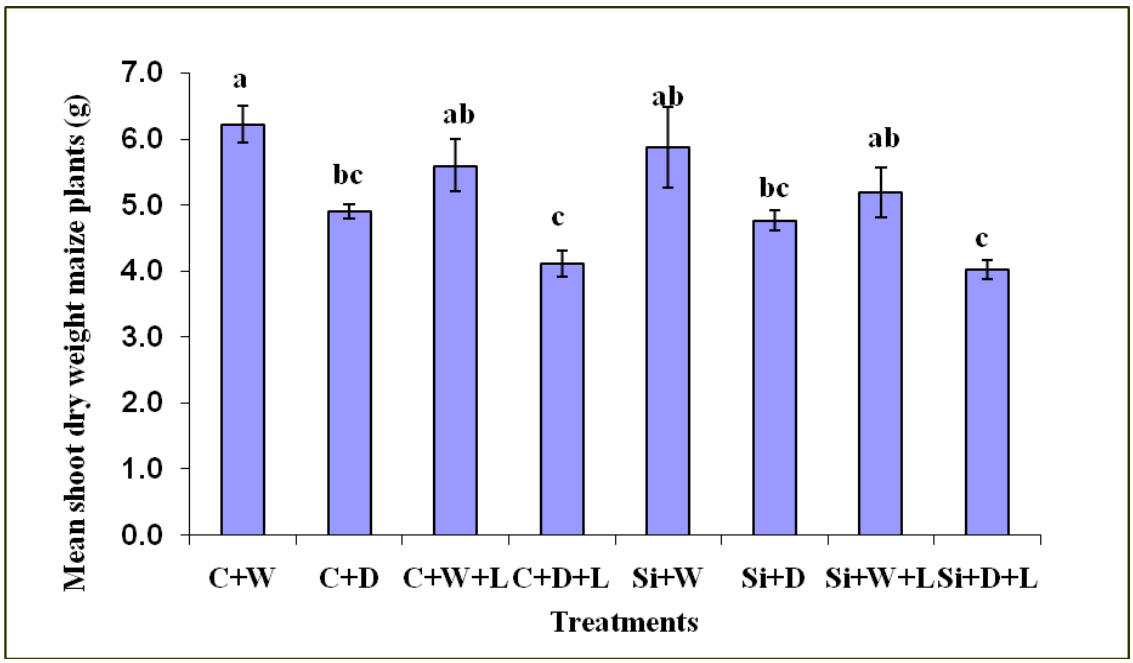


Figure 3.4: Mean (± SE mean) shoot dry weight of *Zea mays* plants in response to silica application treatment, watering treatment, and locust treatment. C = Silica treatment control, Si = Silica solution applied. W = Watering treatment control, D = Drought conditions. L = *S. gregaria* locusts present.

3.4.2 Shoot damage by *Schistocerca gregaria*

A) *Triticum aestivum*

Shoot damage by *S. gregaria* was not significantly affected by the watering treatment, but was significantly reduced by the silica treatment, although there was no significant interaction (Table 3.4). Without silica application, control and water stressed plants received an average of 10.6 % and 8.4 % damage respectively, whereas control and water stressed plants with silicon application received only 4.3 % and 4.7 % damage respectively (Figure 3.5).

***Table* 3.4:** 2-way ANOVA statistical output for overall percentage damage per plant of *T. aestivum* by *S. gregaria*.

Source	DF	SS	MS	F	P
Silica treatment	1	0.036842	0.0368423	23.36	0.000
Watering treatment	1	0.001319	0.0013188	0.84	0.364
Interaction	1	0.001940	0.0019402	1.23	0.272
Error	56	0.088327	0.0015773		

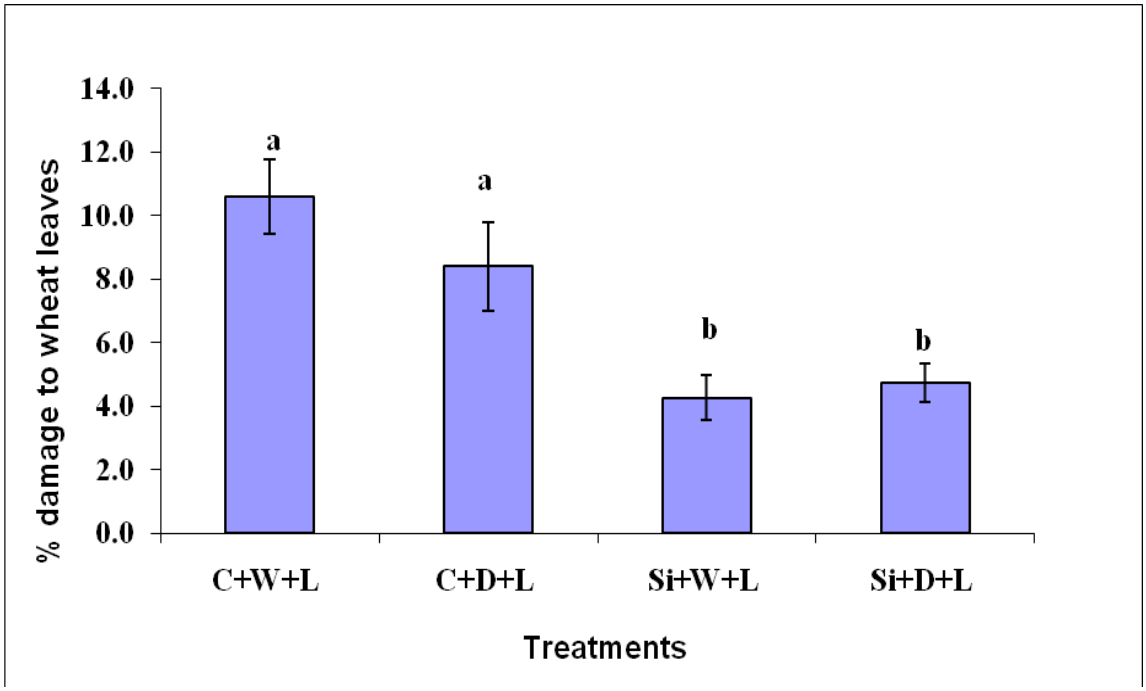


Figure 3.5: Mean (± SE mean) damage of *T. aestivum* leaves in response to silica application treatment, watering treatment, and locust treatment. C = Silica treatment control, Si = Silica solution applied. W = Watering treatment control, D = Drought conditions. L = *S. gregaria* locusts present.

B) *Zea mays*

In *Zea mays,* both the watering treatment and silica solution treatment significantly affected shoot damage by *S. gregaria,* and a significant interaction was seen between these two treatments (Table 3.5). Greater plant damage by *S. gregaria* was observed for the silica treatment control plants, with the water-stressed plants receiving 31.6 % damage and the watering treatment control plants 18.7 % damage (Figure 3.6). However the watering treatment control and water-stressed plants receiving the silica solution received an average of 14.8 % and 15.6 % damage respectively (Figure 3.6).

Table 3.5: 2-way ANOVA statistical output for average percentage damage of *Zea mays* plants by *S.gregaria.*

Source	**DF**	**SS**	**MS**	**F**	**P**
Silica treatment	1	991.58	991.581	12.66	0.001
Watering treatment	1	462.57	462.572	5.90	0.020
Interaction	1	366.07	366.073	4.67	0.037
Error	36	2820.20	78.339		

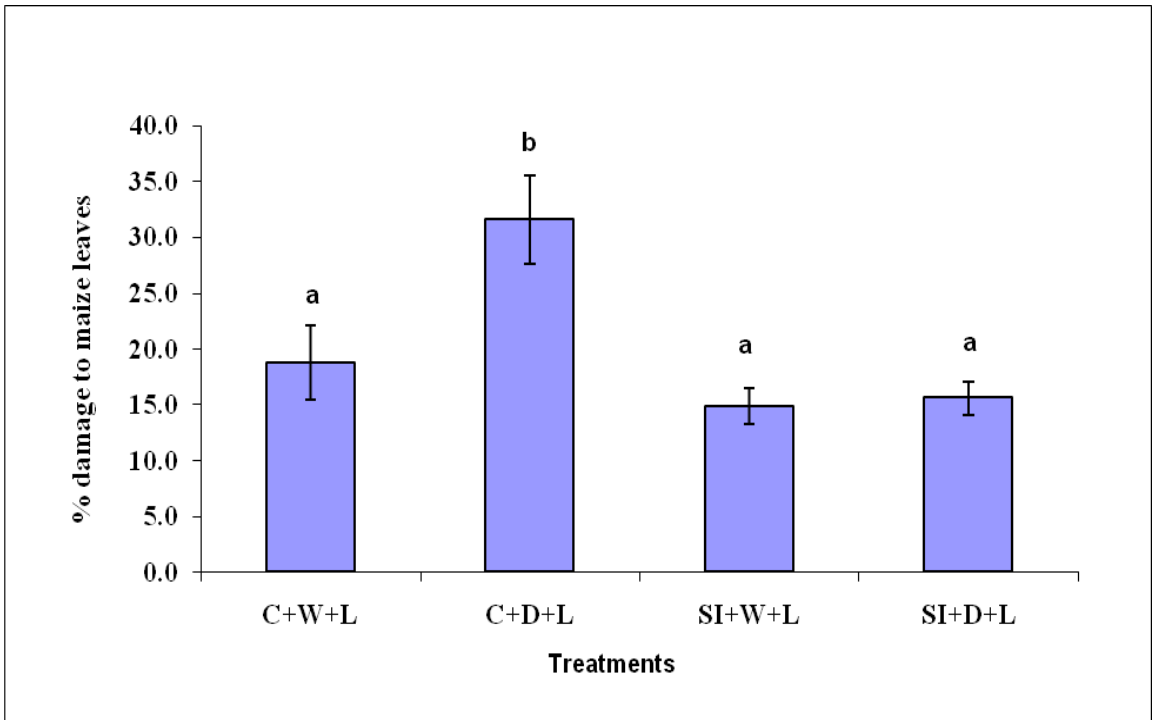


Figure 3.6: Mean (± SE mean) damage percentage of *Z. mays* leaves in response to silica application treatment, watering treatment, and locust treatment. C = Silica treatment control, Si = Silica solution applied. W = Watering treatment control, D = Drought conditions. L = *S. gregaria* locusts present.

In summary, Experiment 1clearly showed that the watering treatment of both *T. aestivum* and *Z. mays* plants, when grown at 40-45 °C, had a significant effect on the shoot biomass. Reduced growth under drought conditions can have a significant detrimental effect on the above ground biomass of both *T. aestivum* and *Z. mays* plants. However, *S. gregaria* herbivory did not show a significant effect on the above ground biomass of *T. aestivum* plants but a significant reduction in shoot biomass was seen for *Z. mays* plants. When silica solution was applied to the roots of both plant species, the shoot biomass of the plants was not significantly affected, regardless of the watering or locust treatments. However, the silica treatment did significantly affect the palatability of both plant species. Silica solution application significantly decreased the percentage damage to the shoots of both plant species. However the palatability of the *Z. mays* shoots was also significantly affected by the watering treatment; unlike *T. aestivum*

plants, drought conditions significantly reduced the percentage of leaves damaged by *S. gregaria* in *Z. mays* plants. Hence the current experimental data clearly shows that the application of silica to plants can significantly reduce plant damage by *S.gregaria*, but this is dependent upon the plant species used and its interaction with the watering treatment.

3.5 Discussion

The experiments in this chapter demonstrate that the application of silica solution to the roots of *T. aestivum* and *Z. mays* affects the palatability of the shoots to the generalist herbivore *S. gregaria.* Under the experimental conditions used, the application of silica solution decreased the amount of shoot damage experienced by both crop species. The application of silicon solution to both crop species did not however affect their shoot biomass over the course of the experiment, compared to the silica treatment control plants. Although the silicon concentration or accumulation in the leaves was not directly measured, the surface of both plant species was rougher to the touch, compared to the silica treatment control plants. Thus it is predicted that the application of silica solution to the soil was incorporated into the shoots of both plant species, and that the increased abrasiveness of the leaves reduced their palatability, leading to the reduced percentage damage. The potential deposition of silicon phytoliths in the lumen of the plant epidermis, in both *T. aestivum* and *Z. mays* may have increased their leaf abrasiveness which could have deterred *S. gregaria* feeding, and also affected the digestion of high-silica leaves.

Massey *et al*. (2006) and Hunt *et al.* (2008) also observed similar results to the experiments presented in this chapter. Both studies found that the addition of silicon to the soil increased the abrasiveness of grass leaves and also deterred shoot feeding by the locust species *S.gregaria*. The increased silicon concentration of the plants could reduce the growth of folivorous insects by reducing the herbivore's metabolic efficiency at converting the plant's leaf mass into its own body mass.

Massey *et al*. (2006) and Hunt *et al.* (2008) suggested that phytoliths produced from silica solution application might be helpful in increasing the strength of chlorenchyma cells, thus reducing the mechanical breakdown of cell walls by chewing herbivores. Additionally the mechanical breakdown of cells could also slow down the rate of digestion in herbivores and deter further feeding, thus reducing herbivore plant damage. Therefore the decreased palatability to *S. gregaria* of *T. aestivum* and *Z. mays* plants treated with silica solution , demonstrated in this chapter's experiments, suggests that silicon application potentially reduces the digestion efficiency of *S. gregaria* due to the reduced mechanical breakdown of cells (Massey *et al.,* 2006; Hunt *et al.* 2008).

Additionally, Cotterill *et al.* (2007) showed that silicon application to the soil increased the abrasiveness of *T.aestivum* leaves and deterred rabbit grazing, thus reducing *T.aestivum* damage and Massey *et al.* (2009) showed that silicon application also affect plant palatability by reducing the bite rate of sheep.

3.6 Conclusion

In conclusion, the results of these studies and the experiments presented in this chapter demonstrate that the application of silica solution to the soil of crop species reduces damage by chewing herbivores, through the increased silica concentration of the plants and their potentially increased abrasiveness. Hence soil silica application is a potential chemical control against chewing herbivores, and did not affect the final shoot biomass of the crop plants, compared to controls.

Chapter 4: Effects of watering treatment and silicon application on *Triticum aestivum* shoot biomass and *Aphis gossypii* performance

4.1 Introduction

The extent to which plants can defend themselves against pest attack and the effectiveness of these defences has been subject to a huge debate in the world of plant science (Reynolds *et al*, 2009), partially due to the variety of herbivore feeding guilds displayed, and also the presence of other abiotic stressors on plant growth such as water stress.

Chapter 3 demonstrated that the soil application of silica solution significantly reduced the palatability of both *T. aestivum* and *Z. mays* to the chewing herbivore *S. gregaria*, and that *S. gregaria* feeding on silica-applied plants had a significantly lower body mass. However, plant defences that are effective against chewing herbivores may not be as effective against phloem feeders, such as aphids.

A. gossypii (Superfamily Aphidoidea, Order Homoptera), is a small, soft-bodied, phloem-sucking bugs and are one of the most harmful crop pests (Dixon, 1985). As stated earlier in General Introduction *A. gossypii* has an extraordinary capacity for population increase and a large feeding capacity and as they can have a direct impact on the physiological process of plants due to the direct insertion of their stylet into the phloem cells, they can cause serious threat to crop production (Patel & Patel., 1997; Goussain *et al*, 2005).

Silicon is a harmless chemical alternative to current chemical pesticides used to control a variety of plant pests (Prabhu *et al*. 2001). Silicon is generally taken up by the plants as silicic acid and in most of the cases, silicon is deposited with in the lumen of epidermal cells, cell walls, intercellular spaces or external layers forming an amorphous hydrated body/ silica gel $SiO_2.nH_2O$, known as phytoliths (Epstein, 1994; Fauteux *et al*., 2005; Massey & Hartley, 2006; Hunt *et al*., 2008).

Goussain *et al*., (2005) and Moraes *et al*., (2005) demonstrated that the longevity and the reproductive stage of the aphid decreased significantly when silicon was applied to the soil and additionally as foliar spray. On the contrary, Massey *et al*., (2006), found that soil silica application did not have any detrimental effects on phloem feeders. Hence few studies have investigated the impact of soil-applied silica solution on plant growth and palatability using phloem feeders, and the effects on the aphid population size when silica is applied to plants is little known. Whereas chapter 3 found that silica application affected plant palatability and the performance of chewing herbivores, silica deposition in plant cells might not provide the same protective effect from phloem-feeding insects, due to the difference in their feeding mechanisms. If deposited phytoliths in a particular part of the plant impedes stylet penetration, phloem feeders could still try to suck sap from another plant part or leaf area that had a lower phytolith concentration, especially if the deposition of silica bodies in the plant cells and the whole plant is not uniform, thus reducing the effectiveness of silica deposition against phloem-feeding insects.

As plants frequently contend with a range of biotic and abiotic stressors, the combined effects of these must also be considered. However few studies have investigated the influence of silica on biotic and abiotic stresses together. By investigating the growth of *T. aestivum* under water-stress and aphid herbivory, we can determine how silica application through the soil may interact with these factors and also affect the performance of *A. gossypii*. *Z. mays* was not investigated in this chapter, as preliminary studies showed that *A. gossypii* had a very low survival on *Zea mays* under the experimental conditions used.

4.2 Chapter aims and hypotheses

4.2.1 Chapter aims

The aim of this chapter was to investigate the effects of root-applied silica solution to *T. aestivum* shoot biomass, when plants were grown under water-stressed conditions (drought-mimicking) in the presence of the phloem-feeding aphid *A. gossypii*, compared to the treatment control plants, and how these factors may interact with each other. Additionally this experiment also investigated the effects of root-applied silica solution on the population size of *A. gossypii,* under each treatment group.

4.2.2 Null hypotheses

1) The shoot biomass of *T. aestivum* when grown at 45 °C is not significantly affected by the watering treatment, silica treatment, or the presence/ absence of *A. gossypii*, compared to the control plants.

2) *A. gossypii population* size is not significantly affected by the watering treatment or silica treatment of the plants they are caged on.

4.3 Methods

4.3.1 Plant growth conditions and treatments

T. aestivum plants were grown and maintained as described in previous chapters. Once plants were established, they were then subjected to one of the eight treatment combinations, each consisting of ten replicates, arranged in a fully randomized block design. The experimental design consisted of a 3-way factorial design, consisting of the treatments ‘Silica Treatment’, ‘Watering Treatment’ and ‘Aphid Treatment’. ‘Silica Treatment’ consisted of the levels ‘Silica Control’ (-Si) and Silicon Application’ (+Si); ‘Watering Treatment’ consisted of the levels ‘Watering Control’ (W) and ‘Drought’ (D), and ‘Aphid Treatment’ consisted of the

levels ‘Aphid Control’ (-L) and ‘Aphid added’ (+L). These treatment-combinations are described below in Table 4.1.

Table 4.1: Design of Experiment 1. ‘Silica Treatment’ consisted of the levels ‘Silica Control’ (-Si) and Silicon Application’ (+Si); ‘Watering Treatment’ consisted of the levels ‘Watering Control’ (W) and ‘Drought’ (D), and ‘Aphid Treatment’ consisted of the levels ‘Aphid Control’ (-A) and ‘Aphid added’ (+A).

Treatment Combination		**Treatment Combination Levels**		
No.	**Code**	**Silica Treatment**	**Watering Treatment**	**Aphid Treatment**
1	C+W	-Si	W	-A
2	C+D	-Si	D	-A
3	C+W+A	-Si	W	+A
4	C+D+A	-Si	D	+A
5	Si+W	+Si	W	-A
6	Si+D	+Si	D	-A
7	Si+W+A	+Si	W	+A
8	Si+D+A	+Si	D	+A

Silicon, watering and drought conditions were as maintained as described in the previous chapters.

Photos of the experimental plants are shown in Figures 3.1 for *T. aestivum*.

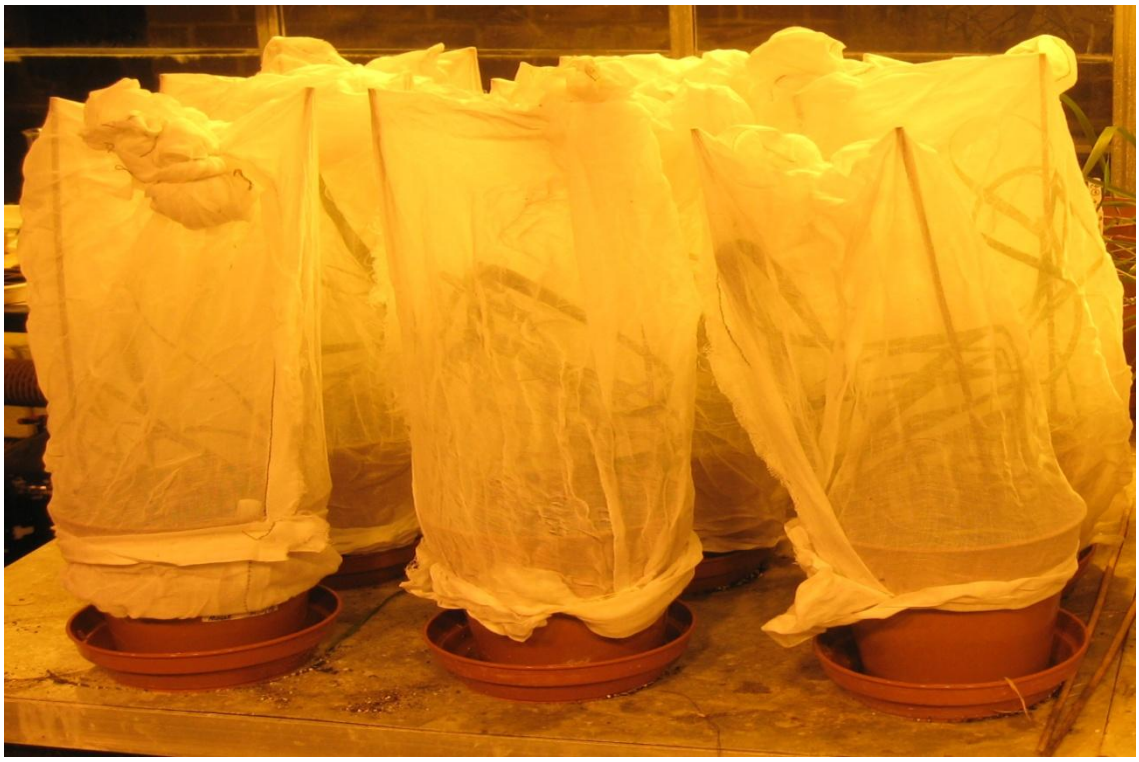


Figure 4.1: Caged *T. aestivum* plants grown under experimental drought conditions and with *A. gossypii* present.

4.3.2 *Aphis gossypii* culture and treatment

A. gossypii were obtained from a single clone from a field site of the University of Sussex campus and were housed in glass cages and maintained on a diet of wheat plants before use. Plants were grown for 45 days under their Watering and Silica treatments before *A. gossypii* were added to ensure that the plants had established enough biomass for the herbivores to consume. Twenty aphids per plant were added in the 'Aphids added' treatment group. All plants in the experiment were covered by 45 cm tall muslin cages under the greenhouse environment with 12 hours full light penetration. Plants were left for a further 15 days before harvesting, to ensure that the aphids had opportunity to feed on the leaves and for their population size to increase.

Figure 4.2: *A. gossypii* on *T. aestivum*

4.3.3 *Aphis gossypii* count estimation

After the experimental period, *A. gossypii* were collected in a 30 ml bottle filled with alcohol by using a soft brush. The total number of *A. gossypii* per plant was estimated by pouring the alcohol-aphid mix into a Petri dish and counting the number of aphids present in a 1 cm^2 area, for 10 replicate areas. This was then extrapolated for the total area of the Petri dish, to determine the total number of aphids on each plant.

4.3.4 Plant harvesting

Plants were harvested after 60 days of growth in their treatment. Shoots and roots were separated using scissors and shoot fresh weights (FW) were recorded for each plant. Plant material was dried at 80°C and subsequently the shoot dry weight (DW) measured.

4.3.5 Data analysis

T. aestivum shoot biomass (DW) data were found to be normally distributed and to have equal variances. Therefore, data were analyzed using a general linear model (GLM), consisting of the factors Silicon Treatment (levels either Control or Silica Applied), Watering Treatment (levels either Watering Control or Drought) and Aphid Treatment (with or without *A. gossypii*). Post-hoc Tukey tests were used to identify the location of significant differences between group means.

A. gossypii count data were found to be normally distributed and to have equal variances. Therefore, data were analyzed using a 2 way ANOVA, consisting of the factors Silicon Treatment (levels either Control or Silica Applied) and Watering Treatment (levels either Watering Control or Drought). Post-hoc Tukey tests were used to identify the significant differences between group means.

4.4 Results

4.4.1 Shoot biomass of Triticum aestivum

The results showed that the shoot biomass (DW) of *T. aestivum* plants grown at 45 °C was significantly affected by the watering treatment and aphid treatment, but the silica solution treatment did not have a significant effect on the shoot biomass, nor were there any significant interactions between the treatments (Table 4.2). Growth under drought conditions and the presence of aphids significantly reduced the shoot biomass of *T. aestivum*, but the application of silica solution did not, compared to the control plants for each treatment (Figure 4.2). The growth of *T. aestivum* under drought conditions significantly reduced the shoot biomass by 40 % compared to the watering treatment control plants, when grown without silica solution. When grown with silica solution, the reduction in biomass from the drought conditions was 33 % lower compared to the control plants. However the silica treatment did not have a significant effect on the size of the difference in shoot biomass of plants grown under contrasting watering treatments (Figure 4.1).

After 60 days, the watering treatment control plants produced an average of 3.5 g shoot biomass and water-stressed plants produced an average of 2 g biomass. The watering treatment control plants produced an average of 2.85 g of biomass in presence of *A. gossypii* (Figure 4.3). When grown without the application of silica solution, the dual stressors of growth under drought conditions and the presence of aphids reduced plant shoot biomass by 46 % compared to the control plants (Figure 4.3).When silica solution was applied, the reduction in shoot biomass caused by the combination of water-stress and the presence of aphids reduced the shoot biomass by a comparatively lower amount of 32 %, although the silica treatment did not have a statistically significant effect on shoot biomass.

Table 4.2: GLM statistical output for shoot biomass of *T. aestivum* plants.

Source	**DF**	**Seq SS**	**Adj SS**	**Adj MS**	**F**	**P**
Silica Treatment	1	0.1666	0.1666	0.1666	0.22	0.642
Watering Treatment	1	27.1573	27.1573	27.1573	35.60	0.000
Aphid Treatment	1	3.3376	3.3376	3.3376	4.37	0.040
Silica Treatment*						
Watering Treatment	1	0.9994	0.9994	0.9994	1.31	0.256
Silica Treatment*						
Aphid Treatment	1	0.9010	0.9010	0.9010	1.18	0.281
Watering Treatment*						
Aphid Treatment	1	0.0882	0.0882	0.0882	0.12	0.735
Silica Treatment*						
Watering Treatment*						
Aphids Treatment	1	0.0001	0.0001	0.0001	0.00	0.990
Error	72	54.9313	54.9313	0.7629		

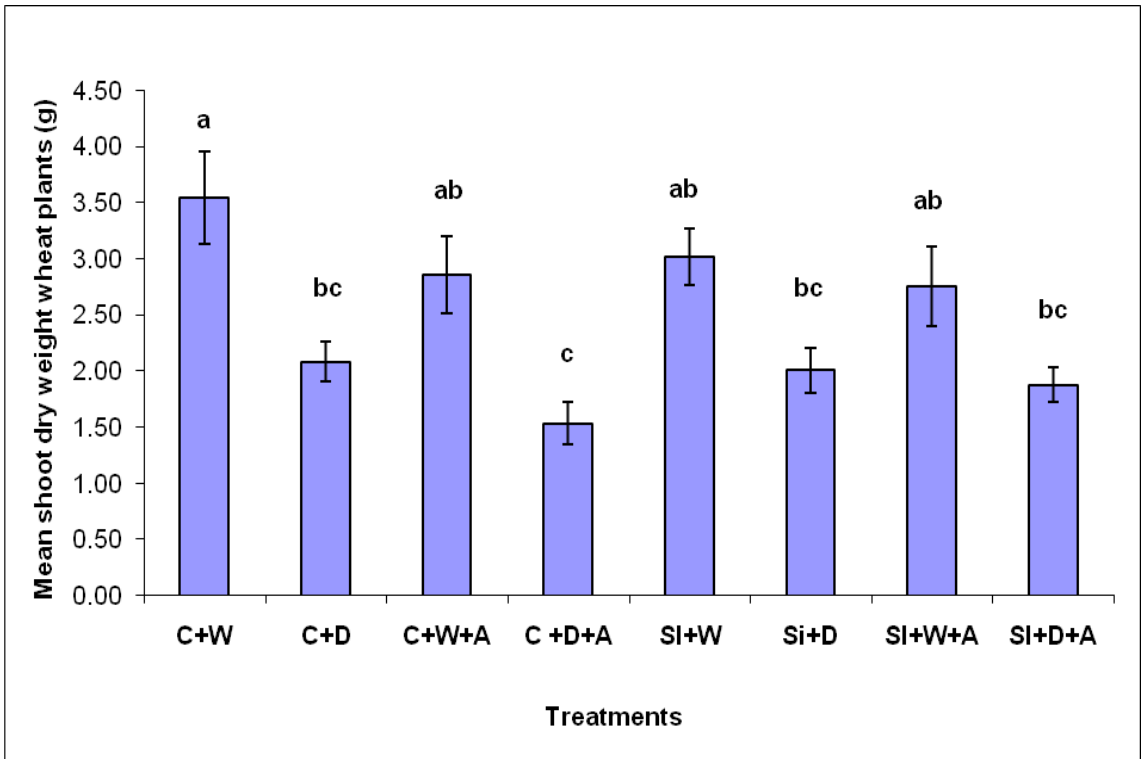


Figure 4.3: Mean (± SE mean) shoot dry weight of *T. aestivum* plants in response to silica application treatment, watering treatment, and *A. gossypoii* treatment. C = Silica treatment control, Si = Silica solution applied. W = Watering treatment control, D = Drought conditions. A = Aphids present.

4.4.2 Aphis gossypii Count

The silica treatment did not show a significant effect on the number of *A. gossypii* on *T. aestivum* plants after 15 days; however the watering treatment did show a significant effect (Table 4.2). Drought conditions generally had a significant effect in reducing the number of aphids compared to the watering treatment control plants (Figure 4.3), but there was a significant interaction between the watering and silica treatments on aphid count (Table 4.2). The aphid count of plants growing without silicon application was slightly reduced by drought conditions, but this was not significant. When grown in the presence of silica, the watering treatment control plants showed the maximum mean

aphid count of 5458, which was significantly reduced to a mean of 1300 under drought conditions (Figure 4.4).

Table 4.3: Statistical output of a 2 way ANOVA on the *Aphis gosypii* count feeding on *T. aestivum*.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Silica Treatment	1	11759659	11759659	11759659	1.56	0.219
Watering Treatment	1	51808758	51808758	51808758	6.89	0.013
Silica Treatment* Watering Treatment	1	35382126	35382126	35382126	4.70	0.037
Error	36	270727412	270727412	7520206		

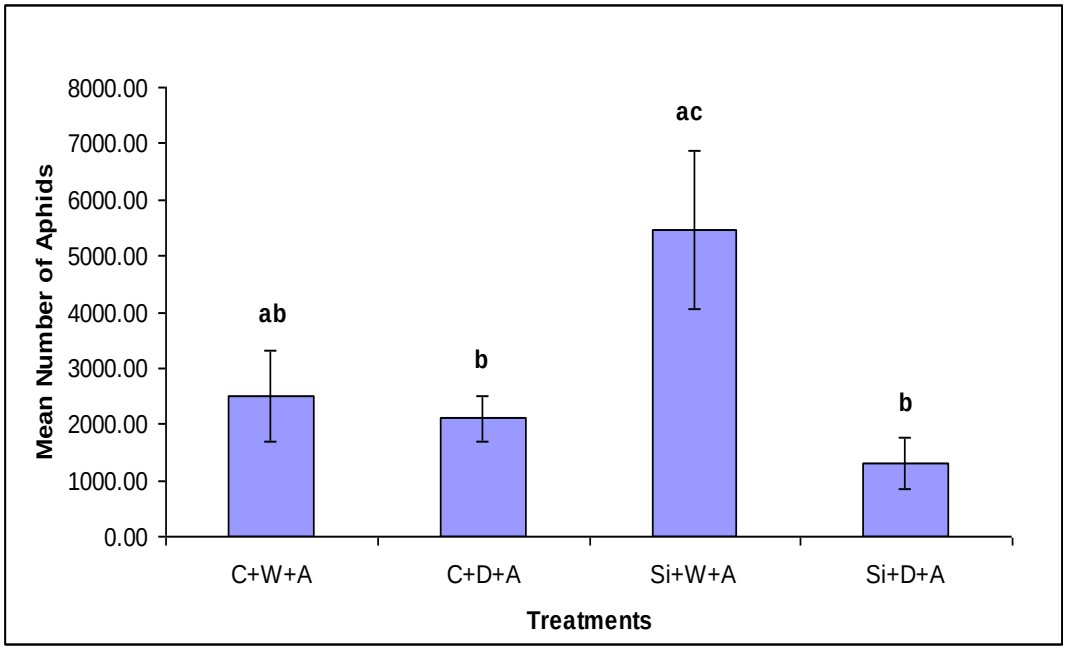


Figure 4.4: Mean (± SE mean) number of *A. gossypii* of *T. aestivum* plants in response to silica treatment and watering application treatment. C = Silica treatment control, Si = Silica solution applied. W = Watering treatment control, D = Drought conditions. A = Aphids present.

In summary, growth under the experimental drought conditions and the presence of aphids can have a significant detrimental effect on the above ground biomass of *T. aestivum* plants. However the application of silica solution did not significantly alter these trends, nor interact with them, when the plants where grown a 45 °C. Additionally, the application of silica did not have a significant effect on the mean *A. gossypii* count. Nevertheless, the slightly reduced aphid count on plants grown under drought conditions, compared to watering treatment control plants, was significantly enhanced under the application of silica solution.

4.5 Discussion

In this study, the soil application of silica solution did not significantly affect the shoot biomass of *T. aestivum* plants, but did significantly interact with the watering treatment to produce the mean highest and lowest aphid counts under the watering treatment control and drought conditions respectively. This suggests that while the application of silica solution alone does not seem to affect aphid count, the effect of silica solution on plants under water-stress can reduced the palatability of the plant to a greater extent to significantly reduce aphid numbers. If the water stress conditions reduced the available plant sap to the aphids, the presence of silica in the plant may reduce the available plant sap further, to have a significantly detrimental effect on aphid numbers. However, to understand the impact of drought and silicon interaction specifically on aphids, further investigation is required.

Massey *et al*. (2006) also showed that the root application of silica solution on grass did not show any detrimental effect on aphid (*S. avenae*) performance, although a significant negative effect was seen in two chewing herbivore species (*S. exempta and S. gregaria*). The authors proposed that silica application and consequential small isolated silica body deposition in the plant epidermis was not able to create a mechanical barrier against aphid stylet penetration.

Although the results presented in this chapter, like those of Massey *et al*. (2006), showed that silica deposition through soil application did not have any detrimental effect on phloem feeders, Goussain *et al*. (2005) and Moraes *et al*. (2005) demonstrated that silica soil application followed by one or two silica foliar sprayings could reduce aphid population size and aphid longevity. Goussain *et al*. (2005) showed that the combined soil and foliar application of silicon to *T. aestivum* decreased the longevity and the reproductive stage of the aphid species *S.graminum*. Moraes *et al*. (2005) also demonstrated that soil-applied silica solution did not affect aphid (*S.graminum*) numbers, but *Zea mays* plants treated with root-applied silicon followed by one foliar silicon spray or two foliar silicon sprays significantly reduced the number of aphids (*S.graminum*).

Goussain *et al*., (2005) suggested that the intracellular accumulation of silica in the tissue spaces and cell wall matrix, and the cell wall deposits of silica, could easily increase the rigidity of cell walls and potentially impede the penetration of the stylet and feeding of phloem-feeding species, through the creation of a mechanical barrier (Goussain *et al*., 2005; Moraes *et al*., 2005). Additionally, increased plant silica concentrations could induce biochemical changes within the plant, causing the stylet to be withdrawn quickly from the plant tissue by diminishing the quality of phloem sap, affecting aphid development (Goussain *et al*., 2005).

4.6 Conclusion

In conclusion, the soil application of silica solution did not have a significant effect on the shoot biomass or *A. gossypii* count. The experiments present in this chapter support the results of Massey *et al*., (2006) and Moraes *et al*., (2005), in that the soil application of silica solution alone does not have a significant effect on aphid numbers. However interaction between silica application and watering treatment suggests that significant plant biochemical and physical changes are produced from the combination of soil application of silica solution and water stress which significantly reduces aphid numbers.

Chapter 5: General Discussion and Conclusions

This thesis concentrated on how silica application to the soil affects two key abiotic and biotic stressors affecting plant growth – the negative influences of water stress and insect herbivory on the shoot growth of two economically important crop species, *T. aestivum* and *Z. mays.* This chapter discusses the key findings of this thesis and its contribution to the scientific literature on the influence of silica on plant-herbivore interactions, and on silica as an economically viable protection against drought.

Key findings

The experiments presented in this thesis have made an important contribution to the understanding of the impact of soil silica application on the shoot growth of the crops *T. aestivum* and *Z. mays* and their interaction with chewing and phloem feeding insects, and with drought conditions.

Chapter 2 specifically investigated the effects of silica application on the shoot biomass and photosynthetic rate of both species under control and drought conditions. Two separate experiments demonstrated that under extreme water stress produced by greenhouse temperatures of 40 – 45 °C and a high perlite ratio, soil silica application had no effect on the shoot biomass of water-stressed plants compared to control plants. However in the second experiment, plants under less extreme drought conditions (produced by lower temperatures of 20-22°C and with a lower perlite ratio), silica application at concentrations of 0.0605 and 0.130 mol/L to the soil significantly increased the shoot biomass and the photosynthetic rate of both species, although this was not dependent upon the silica concentration used. Hence these experiments indicate that the effectiveness of silica solution in protecting plants against water stress may be dependent upon the temperature and/ or growth medium composition, and future experiments should consider these factors when investigating the use of silica solution as a potential agent against drought conditions.

The influence of silica application on plant-herbivore interactions was investigated using two economically important and geographically widespread pest species - the chewing generalist herbivore *S. gregaria* (Chapter 3) and the phloem feeder generalist herbivore *A. gossypii* (Chapter 4). Contrasting effects of silica application to the plant – herbivore interactions were found between these two feeding guilds. Chapter 3 demonstrated that soil silica application significantly decreased the palatability of *T. aestivum* and *Z. mays* to *S. gregaria* compared to control plants, and in a separate experiment, demonstrated that soil silica application also hindered the performance of *S. gregaria,* compared to control plants in no-choice feeding trials. Although the leaves of both *T. aestivum* and *Z. mays* silica-applied plants were observed to be more abrassive to the touch, silica application did not affect the shoot biomass of either crop species compared to the control plants, suggesting that soil silica application may be a viable control agent against chewing generalist pests without affecting shoot biomass. Yet these beneficial effects were not seen under *A. gossypii* herbivory (Chapter 4). Here, soil silica application did not significantly decrease aphid numbers, nor affect the shoot biomass compared to control plants. Thus the use of silica solution as an effective pest control agent, when applied to the soil, will be dependent upon the feeding guild of the pest, although the shoot biomass of *T. aestivum* and *Z. mays* will potentially not be affected.

In summary, the effectiveness of silica application to improve drought tolerance has found to be dependent upon the magnitude of the drought conditions or/and growth medium of plants. Additionally the effectiveness of silica application as a pest control method has been found to be dependent upon the feeding guild of the target herbivore.

5.1 The impact of silica application on *Triticum aestivum* and *Zea mays* plants under drought threat.

Drought is a significantly detrimental environmental factor, reducing crop yield on a global and annual basis (Boyer & Westgate, 2004). For example, in 2011 the most costly drought on record occurred, causing a $5.2 billion agricultural loss in Texas, including water-stress damage to *T. aestivum* ($243 million loss) and *Z. mays* ($327 million loss) (www.fao.org/ , 2011) .Therefore, scientific investigation into increasing the drought tolerance of crops is economically important as well as socially important in preventing crop failure.

Root-applied silica solution is potentially a helpful tool to increase drought tolerance, by increasing the shoot biomass of *T. aestivum* and *Z. mays* under drought conditions compared to control plants. This hypothesis was investigated in chapter 3, and showed that silica application could increase drought tolerance of *T. aestivum* and *Z. mays*, measured by final shoot biomass and photosynthetic rate. However, the magnitude of this beneficial effect depended on the experimental conditions used, such as the water availability (manipulated through temperature and growth medium composition). Thus the beneficial effects of silica application on drought tolerance were not seen under extreme drought conditions, but were seen under moderate drought conditions, suggesting that further research into the interaction between silica application and water availability is required to fully understand the beneficial potential of silica application to drought tolerance.

5.2 The impact of soil silica solution application on the palatability of *Triticum aestivum* and *Zea mays* and the performance of the chewing herbivore *Schistocerca gregaria*.

Locusts, including the desert locust *S. gregaria* are one of the most harmful agricultural pests because of the swarming behaviour produced under high population densities (density dependent polyphenism (Lovejoy *et al*., 2005; Despland *et al*., 2000). More than 60 countries are under threat from the locust swarming behaviour, causing high reduction in agricultural production in Africa, Middle East and Asia over centuries (Dutta et al., 2001). Therefore, scientific research into novel and effective control measures of locust crop damage, and when plants are additionally under water-stress, is required.

The beneficial effects of silica application are not just limited to drought tolerance, but have also been showed to reduce plant palatability against chewing herbivores. Increasing plant silicon concentration through soil silica application can potentially protect against the mechanical breakdown of the plant cell wall by chewing herbivores, by strengthening the chlorenchyma cells through phytolith deposition (Hunt *et al* 2008). Scientific studies have shown that the grass species *Agrostis capillaries* L., *Brachypodium pinnatum* L., *Festuca ovina* L., and *Lolium perenne* L., grown with excess silica application, are more abrasive than these grass species grown without silica application, and the abrasiveness of the leaves is proportional to silica content (Hunt et al., 2008; Massy et al., 2007). Chewing herbivores such as *S. gregaria* have a tendency to choose low silica plants rather than high silica plants and their growth rate is reduced when feeding on high-silica plants in no-choice trails (Massey and Hartley, 2006). This was also demonstrated in the generalist chewing folivores *S. exempta* and *S. gregaria,* where silica application reduced digestion efficiency and also altered the feeding preference between various grass species (Massey *et al*, 2006). Here, the beneficial effect of silica application against *S. gregaria* herbivory was demonstrated in chapter 3 for the cereal crops *T. aestivum* and *Z. mays*, whereby soil silica application

decreased the palatability of both plants and decreased the performance of *S. gregaria* in no-choice feeding trials, without producing detrimental effects to shoot biomass.

5.3 The impact of soil silica solution application to *Triticum aestivum* on the performance of the phloem-feeder *Aphis gossypii*.

Aphids are highly polyphagous insects, widely distributed throughout tropical, subtropical and temperate regions (Satar *et al*, 2005), and cause extremely high plant damage from their high population density (Pinol *et al*, 2009). As a phloem-feeder, they can cause direct damage to the plants by sap ingestion and at the same time they can introduce viruses and other pathogens to the phloem cells, further increasing plant damage (Goussain *et al*, 2005). Chapter 4's results showed that soil silica application did not have any significant effect on *A. gossypii* count compared to control plants and no interaction was seen under drought conditions.

5.4 The potential effects of silica application method on herbivore feeding guild.

Although the results presented in chapter 3 are consistent with studies demonstrating a beneficial role of silica against chewing herbivores in other plant species, the benefits of silica application against phloem-feeders remain controversial. Goussain *et al*., (2005) and Moraes *et al*., (2005) showed that the longevity and the reproductive stage of the aphid *Schizaphis graminum* decreased significantly when silica was applied through a combination of soil and foliar applications, compared to a single application method.

Additionally, Massey *et al*., (2006), demonstrated that soil silicon application did not have any detrimental effect on the phloem feeder *Sitobion avenae*. Chapter 4's results showed that soil silica application did not have any significant effect on the aphid count compared to control plants and no interaction was seen under drought conditions. It

appears that the feeding method of *A. glossypii* through stylet penetration potentially allows for the avoidance of the intra- and extracellular silica bodies that are produced from the uptake of silica from the soil. Liang *et al*. (2005) showed that both foliar spray and root-applied silicon could increase a plant's resistance against pest attack by making a physical barrier or creating natural defences in plants. These authors concluded that foliar spray of silicon could effectively control infections due to producing a physical barrier of deposited silicon on leaf surfaces on plants. Additionally, Guevel *et al*., (2007), proved that foliar application of silicon caused a significant reduction of powdery mildew in wheat plants. Thus further studies investigating the role of silicon in aphid control should investigate different application methods such as foliar sprays and combined foliar and soil application in order to overcome this issue.

5.5 How the photosynthetic pathway type may affect shoot biomass in response to drought conditions and herbivory.

The plant species used in this thesis differ in their photosynthetic pathways, which could have potentially affected their response to the effects of drought and herbivory. *T. aestivum* uses the C3 photosynthetic pathway, whereby single chloroplasts are used to convert light energy to chemical energy. *Z. mays* uses the C4 photosynthetic pathway, converting CO_2 to the four carbon molecule, carbon dicarboxylic acid oxaloacetate, during photosynthesis (Robert *et al*, 1995). These different photosynthetic pathways also affect other aspects of the plant's anatomy, for instance C3 plants contain 50-60 % more protein by dry weight compared to C4 plants (Lyttleton, 1973). Additionally, in C4 plants, the mesophyll cells are arranged around the bundle sheath cells like a garland, known as kranz anatomy (Brown & Smith, 19737). The main reason for this adaptation is to supply CO_2 constantly to the bundle sheath and to overcome the limitation of photorespiration. C4 plants are generally thought to be more drought and pest tolerant plants due to their anatomical structure and because they can reduce photorespiration by accumulation of CO_2 (Robert *et al*, 1995). As bundle sheath cells of C4 plants consist of very thick cell walls, C4 plants are more resistant to physical

disruption, allowing greater mechanical resistance against herbivory, and potentially causing herbivores to attack C3 plants in preference to C4 plant species (Boutton *et al.*, 1978).

In this thesis, growth under drought conditions significantly affected the shoot biomass of both *T. aestivum* and *Z. mays,* but as *Z. mays* (a C4 plant) did not show higher drought tolerance compared to *T. aestivum* (a C3 plant), the photosynthetic pathway may not have influenced the effects of drought on shoot biomass under the experimental conditions used. Additionally, silicon application to soil improved the shoot biomass of both species grown under drought conditions at 20-25^{o}C, suggesting that the effects of silica application on shoot biomass may not depend on the photosynthetic pathway. However, differences in the photosynthetic pathway may have produced certain other differences in the plants' response to drought conditions and soil silica application that were not measured here.

As well as drought resistance, C4 plants are also thought to have greater mechanical resistance to herbivory (Robert *et al*, 1995). Although the C4 plant *Z. mays* did not encourage the growth of *A. gossypii* (causing the experiments in chapter 4 to only use the C3 plant *T. aestivum*), controversial results were seen in chapter 3. Here, *T. aestivum* shoot biomass was not significantly affected by *S. gregaria* herbivory, while *Z. mays* shoot biomass was significantly reduced by presence of *S. gregaria.*

In summary, this thesis did not find that the C4 plant *Z. mays* was more drought tolerant compared to the C3 plant *T. aestivum.* However, differences in herbivory were seen, potentially due to feeding guild, as both C3 and C4 shoot biomass were reduced by *S. gregaria* but C4 plants did not support the growth of *A. gossypii*. As the role of the photosynthetic pathway on herbivory and drought tolerance was not the focus of my thesis, I suggest that future studies investigating the effects of soil silica application to water-stressed plants, and herbivore resistance, also consider that the photosynthetic

pathway may affect the plant's response, potentially in other ways aside from the shoot biomass.

5.6 Conclusions and further research

This thesis has demonstrated that supplemental silicon application to the soil can reduce the negative effects to shoot biomass from drought and herbivory, although this is dependent upon the magnitude of the drought conditions, growth medium of plants and the feeding guild of the herbivores and application method. Since silicon accumulation on plants has been found to have no detrimental impacts on crops and the environment so far (Prabhu *et al*. 2001), it has great potential to be used as a relatively low-cost chemical control measure in agriculture. In this thesis's experiments, 30 ml of 0.0065 mol/L of silica solution applied every other day to *T. aestivum* and *Z. mays* plants was found to produce a 50 % increase in shoot biomass when under drought conditions, compared to the control plants. Additionally the same quantity and concentration reduced plant damage to *T. aestivum* by 60 % and *Z. mays* by 20 %. The low concentration of silica solution required, ease of application and potentially low-environmental effects, makes the application of silica solution a potentially low-cost alternative against drought and chewing herbivore control.

Literature cited

Agarie, S., Hanaoka, N., Ueno, O., Miyazaki, A. K., Kubota, F., Agata, W., & Kaufman, P. B. (1998). Effects of silicon on tolerance to water deficit and heat stress in rice plants (*Oryza sativa L.*), monitored by electrolyte leakage. *Plant production science*, **1**, 96-103.

Barbehenn, R.V., Chen, Z., & Karowe, D. N. (2004). Performance of a generalist grasshopper on a C3 and a C4grass: compensation for the effects of elevated CO_2 on plant nutritional quality. *Oecologia*, **140**, 96–103.

Bi, Y., Tian, S. P., Guo, Y. R., Ge, Y. H., & Qin, G. Z. (2006). Sodium silicate reduces postharvest decay on Hami melons: Induced resistance and fungistatic effects. *Plant disease*, **90**, 279-283.

Borel, W. R., Menzies, J. G., & Belanger, R. R. (2005). Silicon induces antifungal compounds in powdery mildew-infected wheat. *Physiological and Molecular Plant Pathology*, **66**, 108–115.

Boyer, J. S., & Westgate, M. E. (2004). Grain yields with limited water. *Journal of Experimental Botany*, **55**, 2385-2394.

Boutton, T. W., Cameron, G. N & Smith, B. N. (1978). Insect Herbivory on C3 and C4 Grasses. *Oecologia (Berl.)*, **36**, 21 – 32.

Brown, W. V. & Smith, B. N. (1973). The kranz syndrome in plant systematic and evolution. *American journal of botany*, **60**, 34.

Cachorro, P., Ortiz, A., & Cerda, A. (1994). Implications of calcium nutrition on the response of phaseolus-vulgaris L to salinity. *Plant and soil*, **159**, 205-212.

Caswell, H., Reed, F., Stephens, S. N., & Werner, P. A. (1973). Photosynthetic pathways and selective herbivory-hypothesis. *American naturalist*, **107**, 465-480.

Caswell, H. & Reed, F. C. (1975). Indigestibility of C4 bundle sheath-cells by grasshopper. *Entomology*, **68**, 686-688.

Chapman, R. F. (1976). The institute of biologys studies in biology No 71 A biology of locusts. *Institute of Biology's Studies in Biology*, **67**.

Cotterill, J. V., Watkins, R.W., Brennon, C. B. & Cowan, D. P. (2007). Boosting silica levels in wheat leaves reduces grazing by rabbits. *Pest Management Science*, **63**, 247-253.

Datnoff, L. E., Raid, R. N., Synder, G. H., & Jones, D. B. (1991). Effect of calcium silicate on blast and brown spot intensities and yield of rice. *Plant Dis.*, **75**, 729-732.

Davies, W. J., & Zhang, J. H. (1991). Root signals and the regulation of growth and development of plants in drying soil. *Annual review of plant physiology and plant molecular biology*, **42**, 55-76.

Despland, E., Collett, M., & Simpson, S. J. (2000). Small-scale processes in desert locust swarm formation: how vegetation patterns influence gregarization. *Oikos*, **88**, 652–662.

Dixon, A. F. G., & Logan, M. (1973). Leaf size and availability of space to sycamore aphid drepanosiphum platanoides. *Oikos*, **24**, 58-63.

Dixon, A. F. G. (1985). Structure of aphid populations. *Annual review of entomology*, **30**, 155-174.

Dutta, D., Bhatawdekar, S., Chandrasekharan, B., Sharma, J. R., Adiga, S., Wood, D., & McCardle, A. (2001). Desert locust monitoring system –remote sensing and GIS based approach**.** *Satellite Remote Sensing and GIS Applications in Agricultural Meteorology*, 401-423.

Elawad, S.H, & Green, V. E. (1979). Silicon and the rice plant environment: A review of recent research. *Riv.Riso*, **28**, 235-253.

Eneji, A. E., Inanaga, S., An, P., Muranaka, S., Hattori, T., Tsuji, W., & Li, J. (2008). Growth and nutrient use in four grasses under drought stress as mediated by silicon fertilizers. *Journal of Plant Nutrition*, **31**, 355-365.

Epstein, E. (1994). The anomaly of silicon in plant biology. *Proc. Natl. Acad. Sci.*, **91**, 11-17.

Epstein, E. (1999). Silicon. *Plant Physiol. Plant Mol. Biol*, **50**, 641-664.

Epstein, E. (2009). Silicon: its manifold roles in plants. *Annals of Applied Biology*, **10**, 1744-1748.

Farooq, M., Wahid, A., & Siddique, K. H. M. (2009). Advances in drought resistance of rice. *Critical Reviews in Plant Science*, **28**,199-217.

Fauteux, F., Borel, W. R., Menzies, J. G., & Belanger, R. R. (2005). Mini Review, Silicon and plant disease resistance against pathogenic fungi. **249**, 1-6.

Foulkes, M. J., Sylvester-Bradley, R., Weightman, R., & Snape, J. W. (2007). Identifying physiological traits associated with improved drought resistance in winter wheat. *Field crop research*, **103**, 11-24.

Guevel, M. H., Menzies, J. G., & Belanger, R. R. (2007). Effect of root and foliar applications of soluble silicon on powdery mildew control and growth of wheat plants. *Journal of plant pathology*, **119**, 429-436.

Gao, X., Zou, C., Wang, L., & Zhang, F. (2006). Silicon decreases transpiration rate and conductance from stomata of maize plants. *Journal of Plant Nutrition*, **29**, 1637–1647.

Gomes, F. B., Moraes, J. C., Santos, C. D., & Goussain, M. M. (2005). Resistance induction in wheat plants by silicon and aphids. *Sci. Agric. (Piracicaba, Braz.)*, **62**, 547-551.

Gong, H. J, Chen, K. M., Chen, G. C., Wang, S. M., & Zhang, C. L. (2003). Effects of silicon on growth of wheat under drought. *Journal of Plant Nutrition* **26(5)**, 1055-1063.

Gong, H. J., Zhu, X., Chen, K. M., Wang, S. M. & Zhang, C. L. (2005). Silicon alleviates oxidative damage of wheat plants in pots under drought. *Plant Science*. **169**, 313–321.

Goussain, M. M., Pradoi, E., & Moraes, J. C. (2005). Effect of silicon applied to wheat plants on the biology and probing behaviour of the greenbug *Schizaphis graminum*(Rond.) (Hemiptera: Aphididae). *Neotropical Entomology*, **34**, 807-813.

Hattori, T., Inanaga, S., Araki, H., An, P., Morita, S., Luxova, M., & Lux, A. (2004). Application of silicon enhanced drought tolerance in Sorghum bicolor. *Physiologia Plantarum*, **123**, 459-466.

Heidorn, T. & Joern, A. (1984). Differential herbivory on C3 versus C4 grasses by the grasshopper (Orthoptera: acrididae). *Oecologia*, **65**, 19-25.

Hu, Y.C., & Schmidhalter, U. (2005). Drought and salinity: A comparison of their effects on mineral nutrition of plants. *Journal of plant nutrition and soil science*, **168**, 541-549.

Huis, A.V., Cressman, K. & Magor, J.I. (2007). Preventing desert locust plagues: optimizing management interventions. *Entomologia Experimentalis et Applicata*, **122**,191-214.

Hunt, J. W., Dean, A. P., Webster, R. E., Johnson, G. N., & Ennos, A. R. (2008). A Novel mechanism by which silica defends grasses against herbivory. *Annals of Botany*, **102**, 653–656.

Kaufman, P. B., Dayanandan, P., Franklin, C. I., & Takeoka, Y. (1985). Structure and Function of Silica Bodies in the Epidermal System of Grass Shoots. *Annals of Botany*, **55**, 487-507.

Kaya, C.,Tuna, L., & Higgs, D. (2005). Effect of silicon on plant growth and mineral nutrition of maize grown under water-stress conditions. *Journal of Plant Nutrition*, **29**, 1469 – 1480.

Keeping, M. G & Kvedaras, O. L .(2008). Silicon as a plant defense against insect herbivory: response to Massey, Ennos and Hartley. *Journal of Animal Ecology*, **77**, 631-633.

Liang, Y. (1999). Effects of silicon on enzyme activity and sodium, potassium and calcium concentration in barley under salt stress. *Plant and Soil*, **209**, 217–224.

Lovejoy, N. R., Mullen, S. P., Sword, G. A., Chapman, R. F., & Harrison, R. G. (2006). Ancient trans-Atlantic flight explains locust biogeography: molecular phylogenetics of Schistocerca. *The Royal Society*, **273**, 767–774.

Lux, A., Luxova, M., Hattori, T., Inanaga, S., & Sugimoto, Y. (2002). Silicification in sorghum (Sorghum bicolor) cultivars with different drought tolerance. *Physiologia plantarum*, **115**, 87-92.

Ma, J. F. & Yamaji, N. Review: Functions and transport of silicon in plants. (2008).*Cell. Mol. Life Sci.*, **65**, 3049-3057.

Ma, J. F., Goto, S., Tamai, K., & Ichii, M. (2001). Role of root hairs and lateral roots in silicon uptake by rice. *Plant Physiology*, **127**, 1773-1780.

Massey, F. P., Ennos, A. R., & Hartley, S. E. (2006). Silica in grasses as a defence against insect herbivores: contrasting effects on folivores and a phloem feeder. *Journal of Animal Ecology*, **75**, 595-603.

Massey, F. P., Ennos, A. R., & Hartley, S. E. (2007). Herbivore specific induction of silica-based plant defences. *Oecologia*, **152**, 677-683.

Massey, F. P., Ennos, A. R., & Hartley, S. E. (2007). Grasses and the resource availability hypothesis: the importance of silica-based defences. *Journal of Ecology*, **95**, 414-424.

Massey, F. P., Massey, K., Ennos, A. R., & Hartley, S. E. (2009). Impacts of silica-based defences in grasses on the feeding preferences of sheep. *Basic and Applied Ecology*, **10**, 622–630.

Mayland, H. F., Wright, J. L., & Sojka, R. E. (1991). Silicon accumulation and water uptake by wheat. *Plant and soil*, **137**, 191-199.

Mecfel, J., Hinke, S., Goedel, W.A., Marx, G., Fehlhaber R., & Wienhaus, O. (2007). Effect of silicon fertilizers on silicon accumulation in wheat. *J. Plant Nutrition*, **170**, 769-772.

Minks, A. K., & Harrewijn, P. (1988). World crop pests **Vol. 2B**. Aphids their biology natural enemies and control. *World Crop Pests*, XIX+364P.

Mitani, N., Yamaji, N., & Ma, J. F. (2009). Identification of maize silicon influx transporters. *Plant cell physiology*, **50**, 5-12.

Moraes, J. C., Goussain, M. M., Carvalho, G. A., & Costa, R. R. (2005). Feeding non-pre ference of the corn leaf aphid *Rhopalosiphum maidis* (Fitch, 1856) (Hemiptera: Aphididae) to corn plants (*Zea mays L.*), **29**, 761-766.

Moraes, J. C., Goussain, M. M., Basagli, M. A. B., Carvalho, G. A., Ecole, C. C., & Ampaio, M. V. S. (2004). Silicon influence on the tritrophic interaction: wheat plants, the greenbug Schizaphis graminum (Rondani) (Hemiptera: Aphididae), and its natural enemies, Chrysoperla externa (Hagen) (Neuroptera: Chrysopidae) and Aphidius colemani Viereck (Hymenoptera: Aphidiidae). *Neotropical Entomology*, **33(5)**, 619-624.

Nogues, S., & Baker, N. R. (2000). Effects of drought on photosynthesis in Mediterranean plants grown under enhanced UV-B radiation. *Journal of experimental botany*, **51**, 1309-1317.

Pandey, D. N., Chaubey, A. C., Gupta, A. K., & Vardhan, H. (2005). Mine spoil restoration: a strategy combining rainwater harvesting and adaptation to random recurrence of droughts in Rajasthan. International forestry review, **7**, 241-249.

Patel, M. G., & Patel, J. R. (1997). Relation between insect damage and yield of Indian mustard (Brassica juncea) sown on different dates in Gujarat. *Indian Journal of Agricultural Sciences*, **67**, 157-158.

Pei, Z. F., Geng, X. X., Ming, D. F., Gong, H. J., Liu, D., Wan, G. L., & Zhou,W. J. (2010). Silicon improves the tolerance to water-deficit stress induced by polyethylene glycol in wheat (Triticum aestivum L.) seedlings. *J.of Plant Growth Regul*, **29**, 106-115.

Pedgeley, D. E. (1983). Meterology and desert locust forecasting. *OEPP*, **13**, 241-244.

Prabhu, A. S., Barbosa filho, M. P., Filippi , M. C. ,Datnoff, L. E. & Sndyer, G. H. (2001). Silicon from rice disease control perspective in Brazil. In: Datnoff, L.E., Snyder, G.H. & Korndorfer, G.H. (Eds.) Silicon in Agriculture. *Studies in Plant Science, Elsevier Science B. V.*, Amsterdam, The Netherlands, **8**, 293-311.

Rafi, M. M., & Epstein, E. (1999). Silicon absorption by wheat (*Triticum aestivum L.*). *Plant and Soil*, **211**, 223–230.

Rains, D. W., Epstein, E., Zasoski, R. J., & Aslam, M. (2006). Active silicon uptake by wheat. *Plant and Soil*, **280**, 223-228.

Reynolds, O. L., Keeping, M. G., Meyer, J. H. (2009). Silicon-augmented resistance of plants to herbivorous insects: a review. *Annals of applied biology*, **155**, 171-186.

Robert, T. F., & Wllliam. C. T. (1995). Regulation of photosynthesis in C3 and C4 Plants: a molecular approach. *The Plant Cell*, **7**, 797-807.

Rodrigues, F. A., McNally, D. J., Datnoff, L. E., Jones, J. B., Labbe, C., Benhamou, N., Menzies, J. G., & Belanger, R. R. (2003). Silicon enhances the accumulation of Diterpenoid Phytoalexins in rice: a potential mechanism for blast resistance. *The American Phytopathological Society*, **94**, 177-183.

Sanchez-Zapata, J. A., Donazar, J. A., Delgado, A., Forero, M. G., Ceballos, O. & Hiraldo, F. (2007). Desert locust outbreaks in the Sahel: resource competition, predation and ecological effects of pest control. *Journal of Applied Ecology*, **44**, 323-329.

Satar, S., Kersting, U., & Uygun, N. (1998). Development and fecundity of Aphis Gossypii Glover (Homoptera: Aphididae) on three malvaceae hosts. *J. of Agriculture and Forestry*, **23**, 637-643.

Satar, S., Kersting, U., & Uygun, N. (2005). Effect of temperature on development and fecundity of Aphis gossypii Glover (Homoptera : Aphididae) on cucumber. *Journal of Pest Science*,**78**, 133-137.

Savant, N. K., Korndorfer, G. H., Datnoff, L. E., & Snyderc, G. H. (1999). Silicon nutrition and sugarcane production: A Review. *Journal of Plant Nutrition*, **22(12)**, 1853-1903.

Simpson, S. J., McCaffery, A. R., & Hagele, B. F. (1999). A behavioural analysis of phase change in the desert locust. *Biologiocal Reviews of the Cambridge Philosophical Society*, **74**, 461-480.

Simpson, S. J., Despland, E., Hagele, B. F., & Dodgson, T. (2001). Gregarious behavior in desert locusts is evoked by touching their back legs. *PNAS*, **98**, 3895-3897.

Solomon, M. G., Cross, J. V., Fitzgerald, J. D., Campbell, C. A. M., Jolly, R. L., Olszak, R.W., E. Niemczyk, E. & Vogt, H. (2000). Biocontrol of pests of apples and pears in Northern and Central Europe. 3. Predators. *Biocontrol Science Technology*, **10**, 91–128.

Sivamani, E., Bahieldin, A., Wraith, J. M., Al-Niemi, T., Dyer, W. E., David Ho, T-H., & Qu, R. (2000). Improved biomass productivity and water use efficiency under water deficit conditions in transgenic wheat constitutively expressing the barley HVA1 gene. *Plant Science*, **155**, 1–9.

Sunderland, K. D. (1988). Carabidae and other invertebrates. In Minks A.K. & Harrewijn P. (eds): *Aphids, their Biology, Natural Enemies and Control*. **Vol. 2B**. Elsevier, Amsterdam, 293–310.

Uvarov, B. (1977). Grasshoppers and locusts: A handbook of general Acridology **Vol. 2**; behavior ecology bio geography population dynamics, 613.

Van der Werf, W., Woldewahid, G., Van Huis, A., Butrous, M., & Sykora, K. (2005). Plant communities can predict the distribution of solitarious desert locust Schistocerca gregaria. *Journal of applied ecology*, **42**, 989-997.

White, T. C. R. (1976). Weather, food and plagues of locusts. *Oecologia (Berl.)*, **22**, 119-134.

Woldewahid, G., Werf, W. V. D., Huis, A.V. & Stein, A.(2004). Spatial distribution of populations of solitarious adult desert locust (Schistocerca gregaria Forsk.) on the coastal plain of Sudan. *Agricultural and Forest Entomology*, **6**, 181-191.

Yamaji, N., Mitatni, N. & Ma, J. F. (2008). A Transporter regulating silicon distribution in rice shoots. *The Plant Cell*, **20**, 1381–1389.

Zhang, S. Z., Cazelles, B., Tian, H., Stige, L. C., Bräuning, A. & Chrdoi, N. (2009). Plagues in China Periodic temperature-associated drought/flood drives locust. **1284**, 823-831.

http://www.fao.org/news/story/en/item/53813/icode/ 23rd March, 2011.

http://www.topcommodities.net/2011/04/igc-forecast-world-maize production.html/11th April, 2012.

www.fao.org,/23rd March, 2011.